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Ambient Water Quality Criteria for Carbon Tetrachloride

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16. ABSTRACT

Section 304(A) of the Clean Water Act [33 U.S.C. 1314(A)], requires EPA to publish and periodically update water quality criteria. These criteria are to reflect the latest scientific knowledge on the identifiable effects of pollutants on public health and welfare, aquatic life, and recreation. This report presents water quality criteria for Carbon Tetrachloride. It presents concentration criteria for the protection of freshwater and saltwater aquatic life. It presents 'safe' concentrations for humans, and in the case of suspect or proven carcinogens, gives various levels of incremental cancer risk. A Section 304(A) water quality criterion is a qualitative or quantitative estimate of the concentration of a water constituent or pollutant in ambient waters which, when not exceeded, will ensure a water quality sufficient to protect a specified water use. Under the Act a criterion is a scientific entity, based solely on data and scientific judgment. It does not reflect considerations of economic or technological feasibility nor is it a water quality standard and in itself has no regulatory effect.

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AMBIENT WATER QUALITY CRITERIA FOR
CARBON TETRACHLORIDE

Prepared By
U.S. ENVIRONMENTAL PROTECTION AGENCY

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FOREWORD

Section 304 (a)(1) of the Clean Water Act of 1977 (P.L. 95-217), requires the Administrator of the Environmental Protection Agency to publish criteria for water quality accurately reflecting the latest scientific knowledge on the kind and extent of all identifiable effects on health and welfare which may be expected from the presence of pollutants in any body of water, including ground water. Proposed water quality criteria for the 65 toxic pollutants listed under section 307 (a)(1) of the Clean Water Act were developed and a notice of their availability was published for public comment on March 15, 1979 (44 FR 15926), July 25, 1979 (44 FR 43660), and October 1, 1979 (44 FR 56628). This document is a revision of those proposed criteria based upon a consideration of comments received from other Federal Agencies, State agencies, special interest groups, and individual scientists. The criteria contained in this document replace any previously published EPA criteria for the 65 pollutants. This criterion document is also published in satisifaction of paragraph 11 of the Settlement Agreement in Natural Resources Defense Council, et. al. vs. Train, 8 ERC 2120 (D.D.C. 1976), modified, 12 ERC 1833 (D.D.C. 1979).

The term "water quality criteria" is used in two sections of the Clean Water Act, section 304 (a)(1) and section 303 (c)(2). The term has a different program impact in each section. In section 304, the term represents a non-regulatory, scientific assessment of ecological effects. The criteria presented in this publication are such scientific assessments. Such water quality criteria associated with specific stream uses when adopted as State water quality standards under section 303 become enforceable maximum acceptable levels of a pollutant in ambient waters. The water quality criteria adopted in the State water quality standards could have the same numerical limits as the criteria developed under section 304. However, in many situations States may want to adjust water quality criteria developed under section 304 to reflect local environmental conditions and human exposure patterns before incorporation into water quality standards. It is not until their adoption as part of the State water quality standards that the criteria become regulatory.

Guidelines to assist the States in the modification of criteria presented in this document, in the development of water quality standards, and in other water-related programs of this Agency, are being developed by EPA.

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CRITERIA DOCUMENT

CARBON TETRACHLORIDE

CRITERIA

Aquatic Life

The available data for carbon tetrachloride indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 35,200 µg/l and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of carbon tetrachloride to sensitive freshwater aquatic life.

The available data for carbon tetrachloride indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 50,000 µg/l and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of carbon tetrachloride to sensitive saltwater aquatic life.

Human Health

For the maximum protection of human health from the potential carcinogenic effects due to exposure of carbon tetrachloride through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero based on the non-threshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 4.0 µg/l, 0.40µg/l, and 0.04 µg/l, respectively. If the above estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 69.4 µg/l, 6.94 µg/l, and 0.69 µg/l respectively.

INTRODUCTION

Carbon tetrachloride (CCl_4) is a haloalkane with a wide range of industrial and chemical applications. The chemical is also known as tetrachloromethane and perchloromethane. Approximately 423,000 metric tons (932.7 million pounds) are produced each year at 11 plant sites in the U.S. (U.S. EPA, 1977; John, 1976). Most of this chemical is used in the manufacture of fluorocarbons (95 percent in 1973), which were once used primarily as aerosol propellants. Carbon tetrachloride is used as a component of fire extinguisher solutions and as an industrial and chemical solvent. Its use in grain fumigation is being largely replaced by other registered pesticide products. Its use as a degreaser in the dry-cleaning industry has been largely replaced by perchloroethylene (Johns, 1976).

Carbon tetrachloride has a molecular weight of 153.82, a melting point of -22.99°C, and a boiling point of 76.54°C (Weast, 1972). It is a heavy (density of 1.594 g/ml), colorless liquid at room temperature (Hardie, 1964). The compound is relatively nonpolar and miscible with alcohol, acetone, and most other organic solvents. Its solubility in water at 25°C is 800,000 µg/l, and its vapor pressure at 10°C is 55.65 mm Hg (Hardie, 1964). It has an octanol/water partition coefficient of 2.73 (U.S. EPA, 1978).

Carbon tetrachloride may be quite stable under certain environmental conditions. An estimated 70,000 years are required for half of a given quantity of CCl_4 to decompose in water (Johns, 1976). This decomposition rate is considerably accelerated in the presence of metals such as iron (Pearson and McConnell, 1975). Hydrolytic decomposition as a means of removal from water appears to be insignificant as compared to evaporation. Dilling, et al. (1975) determined that CCl_4 has an evaporative half-life of 29 minutes in water at ambient temperatures.

Volatilization is the major transport process for removal of tetrachloromethane from aquatic systems. Once in the troposphere, tetrachloromethane remains stable; it exhibits an extremely slow rate of reaction with hydroxyl radicals present in the troposphere. Tetrachloromethane eventually diffuses into the stratosphere or is carried back to the earth during the precipitation process. Once in the stratosphere, tetrachloromethane is degraded on exposure to shorter wavelength, higher energy ultraviolet light to eventually form phosgene as the principal initial product (U.S. EPA, 1979).

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Aquatic Life Toxicology*

INTRODUCTION

The majority of the acute toxicity data for carbon tetrachloride and aquatic organisms has been determined using static procedures with unmeasured test concentrations. Results of these tests may underestimate the acute toxicity of carbon tetrachloride due to its volatility. No acute or chronic effects were observed at a concentration lower than 3,400 µg/l.

EFFECTS

Acute Toxicity

The 48-hour EC_{50} is 35,200 µg/l for Daphnia magna (Table 1). The bluegill has been tested (Dawson, et al. 1977, U.S. EPA, 1978) and the 96-hour LC_{50} values are 125,000 and 27,300 µg/l, respectively (Table 1). The reason for this large difference is not clear, but may have been caused by the volatility of this compound. There appears to be no great difference in sensitivity between the two tested species. A flow-through test result for the fathead minnow is 43,100 µg/l. However, no comment can be made concerning the effect of test conditions on test results.

Only two saltwater fish and no invertebrate species have been tested and the 96-hour LC_{50} for the tidewater silversides is 150,000 µg/l (Table 1). The other datum is an estimated 96-hour LC_{50} for the dab of about 50,000 µg/l (Table 4).

Chronic Toxicity

No chronic test has been conducted with a freshwater invertebrate species or any saltwater species. An embryo-larval test with the fathead minnow (U.S. EPA, 1978) was conducted, and no adverse effect was observed at carbon tetrachloride concentrations up to 3,400 µg/l (Table 2).

---

*The reader is referred to the Guidelines for Deriving Water Quality Criteria for the Protection of Aquatic Life and Its Uses in order to better understand the following discussion and recommendation. The following tables contain the appropriate data that were found in the literature, and at the bottom of each table are calculations for deriving various measures of toxicity as described in the Guidelines.

Plant Effects

There are no data describing the effects of carbon tetrachloride on any alga or aquatic plant.

Residues

The bluegill bioconcentrated carbon tetrachloride at equilibrium to a factor of 30 times within 21 days (Table 3). The biological half-life in these tissues was less than 1 day. In addition, Neely, et al. (1974) exposed the rainbow trout to carbon tetrachloride and estimated a steady-state bioconcentration factor of 17 (Table 4). These results indicate that tissue residues of carbon tetrachloride should not pose a potential environmental hazard to aquatic life.

Summary

Only two freshwater fish and one invertebrate species have been acutely tested and a 96-hour LC_{50} has been determined as low as 27,300 µg/l. No definitive chronic data are available. Tissue residues of carbon tetrachloride do not appear to be a problem since available data suggest a bioconcentration factor of less than 30.

CRITERIA

The available data for carbon tetrachloride indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 35,200 µg/l and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of carbon tetrachloride to sensitive freshwater aquatic life.

The available data for carbon tetrachloride indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 50,000 µg/l and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of carbon tetrachloride to sensitive saltwater aquatic life.

Table 1. Acute values for carbon tetrachloride

Species	Method*	LC50/EC50 (µg/l)	Species Acute Value (µg/l)	Reference
		FRESHWATER SPECIES		
Cladoceran, *Daphnia magna*	S, U	35,200	35,200	U.S. EPA, 1978
Fathead minnow, *Pimephales promelas*	FT, M	43,100	43,100	Kimball, manuscript
Bluegill, *Lepomis macrochirus*	S, U	125,000	-	Dawson, et al. 1977
Bluegill, *Lepomis macrochirus*	S, U	27,300	58,000	U.S. EPA, 1978
		SALTWATER SPECIES		
Tidewater silversides, *Menidia beryllina*	S, U	150,000	150,000	Dawson, et al. 1977

* S = static, FT = flow-through, U = unmeasured, M = measured

No Final Acute Values are calculable since the minimum data base requirements are not met.

Table 2. Chronic values for carbon tetrachloride (U.S. EPA, 1978)

Species	Method*	Limits (µg/l)	Chronic Value (µg/l)
	FRESHWATER SPECIES		
Fathead minnow, *Pimephales promelas*	E-L	>3,400	>3,400

---

* E-L = embryo-larval

No acute-chronic ratio is calculable.

Table 3. Residues for carbon tetrachloride (U.S. EPA, 1978)

Species	Tissue	Bioconcentration Factor	Duration (days)
	FRESHWATER SPECIES		
Bluegill, *Lepomis macrochirus*	whole body	30	21

Table 4. Other data for carbon tetrachloride

Species	Duration	Effect	Result (µg/l)	Reference
		FRESHWATER SPECIES		
Rainbow trout, *Salmo gairdneri*	-	Estimated steady-state bioconcentration factor = 17	-	Neely, et al. 1974
		SALTWATER SPECIES		
Dab, *Limanda limanda*	96 hrs	LC50	ca. 50,000	Pearson & McConnell, 1975

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Mammalian Toxicology and Human Health Effects

EXPOSURE

Humans are exposed to carbon tetrachloride (CCl_4) and other nonfluorinated halomethanes by three primary routes: intake of water and other fluids, inhalation, and ingestion of foodstuffs (National Research Council (NRC), 1978). Of all of the nonfluorinated halomethanes, only carbon tetrachloride and chloroform have been observed or studied extensively in all three of these primary routes. It is not known if humans absorb CCl_4 more efficiently when exposed via one of these routes versus another. However, it appears as though inhalation is generally the most important route of entry (NRC, 1978). Absorption through the skin is a common route in occupational exposures. The National Institute for Occupational Safety and Health (NIOSH) estimates that 160,000 people are potentially exposed to carbon tetrachloride in their working environment (NIOSH, 1975).

In the early 1920's, CCl_4 was used as an effective oral anthelmintic (NIOSH, 1975; Hall, 1921a,b). This is the only known intentional oral exposure aside from suicidal attempts.

Ingestion from Water

Carbon tetrachloride has been found in many sampled waters (including rain, surface, potable, and sea) in the sub-part per billion (sub-ppb) range (McConnell, et al. 1975). Low levels have even been detected in snow. In the National Organic Reconnaissance Survey (NORS) of 80 cities CCl_4 was found in 10 percent of the U.S. drinking water supplies at levels less than 2 to 3 µg/l (Kopfler, et al. 1976). In New Orleans, CCl_4 was found in drinking water. However, CCl_4 was found in only four samples of raw water with a maximum concentration of 4 µg/l. Confirmatory analyses performed by an alternate analytical technique indicated a level of 2 µg/l.

A more recent drinking water study, the National Organics Monitoring Survey (NOMS), sampled 113 public water systems and found carbon tetrachloride at very low concentrations relative to levels of chloroform and other organics (U.S. EPA, 1977). Positive results were noted in about 10 percent of the samples with mean values ranging from 2.4 to 6.4 µg/l depending on sampling and analytical procedures.

Carbon tetrachloride is a chlorinated hydrocarbon. However, unlike other members of this group of organic chemicals, CCl_4 is not produced in finished drinking water as a result of the chlorination process (NRC, 1977, 1978).

Ingestion from Food

Carbon tetrachloride has been detected in a variety of foodstuffs other than fish and shellfish in levels ranging from 1 to 20 µg/kg. A rough summation of the different food categories that have been shown to be particularly susceptible to CCl_4 contamination follows (McConnell, et al. 1975).

dairy	0.2-14.0 µg/kg
meat	7.0- 9.0 µg/kg
oils and fats	0.7-18.0 µg/kg
beverages	0.2- 6.0 µg/kg
fruits and vegetables	3.0- 8.0 µg/kg
black grapes (imported)	19.7 µg/kg
fresh bread	5.0 µg/kg

Although the highest amount of CCl_4 detected in food has been 19.7 ug/kg in imported black grapes, the concentration permitted by the FAO/WHO expert committee is 50 µg/kg for cooked cereal products (NRC, 1978). McConnell, et al. (1975) note that there is no evidence of significant bioaccumulation of CCl_4 via the food chain to higher trophic levels.

Carbon tetrachloride is used as a food fumigant either alone or in admixture with ethylene dichloride (EDC), ethylene dibromide (EDB), methyl bromide (MB), and/or other solvents. Some common commercial mixtures cited by a National Research Council report (1978) are given in Table 1.

Residues of CCl_4 have been found in commercially fumigated wheat, corn, and milo in amounts ranging from 2.9 to 20.4 mg/kg after 1 to 3 hours of storage (McMahon, 1971). Residues have also been detected in food products containing grain. From 1964 to 1966, Wit (1972) analyzed a number of samples of cereals imported into the Netherlands. Carbon tetrachloride residues ranged from 0.1 to 1.0 mg/kg in 20 percent of the samples, 0.5 to 1.0 mg/kg in 5 percent of the samples, 1.0 to 5.0 mg/kg in 8 percent of the

TABLE 1

Commercial Mixtures of Fumigants Containing Carbon Tetrachloride*

Mixture Number	Chemical Contents	Amounts (%)
1	ethylene dichloride (EDC)	75
	carbon tetrachloride (CCl_4)	25
2	carbon tetrachloride (CCl_4)	80
	carbon disulfide (CS_2)	20
3	carbon tetrachloride (CCl_4)	60
	ethylene dichloride (EDC)	35
	ethylene dibromide (EDB)	5
4	trichloroethylene ($CHCl{=}CCl_2$)	64
	carbon disulfide (CS_2)	26
	carbon tetrachloride (CCl_4)	10
5	chloroform ($CHCl_3$)	37
	trichloroethylene ($CHCl{=}CCl_2$)	32
	carbon disulfide (CS_2)	26
	carbon tetrachloride (CCl_4)	5

*Source: Bielorai and Alumot, 1966

samples, and greater than 5.0 mg/kg in 3 percent of the samples. The maximum amount of CCl_4 residue found was 58 mg/kg (NRC, 1978.)

Commercially fumigated samples of flour contained CCl_4 residues from 0.2 to 0.3 mg/kg (Bondi and Alumot, 1972). Bread and biscuits made from this same flour were free of any detectable amounts of CCl_4 (i.e., less than 0.005 mg/kg).

Through laboratory experiments, researchers have managed to simulate commercial fumigation conditions. Results similar to those obtained from tests performed on commercially fumigated commodities have been obtained. A report by the National Research Council (1978) contains a discussion of such laboratory studies that have been performed to date. The summary of this report is included in the following discussion.

Wit, et al. (1972) analyzed 75 kg sacks of wheat that were fumigated with a mixture of CCl_4-EDC-EDB (10.2:8:1 by weight) and then were aerated for several weeks. Carbon tetrachloride residues within the sacks ranged from 20 to 62 mg/kg. White flour processed from this wheat had residues ranging from 2 to 10 mg/kg, and bread from this wheat had residues up to 0.007 mg/kg.

Scudamore and Heuser (1973) analyzed wheat and corn samples following 3 to 6 days of application by vaporization of a weighed amount of CCl_4 sufficient to give an average vapor concentration of 80 mg/l air. The initial residues ranged from 200 to 400 mg/kg. After six months of aeration the residues ranged from 1 to 10 mg/kg. Residues up to 4.7 mg/kg were found in whole kernel wheat after 12 months aeration, indicating that carbon tetrachloride residues can be very persistent.

Bielorai and Alumot (1966) analyzed wheat and barley following CCl_4 treatment at 40 gm of commercial mixture No. 5 (Table 1) per m^3 of air for 72 hours. The initial residues of 1.53 and 2.2 mg/kg in wheat and barley, respectively, decreased to 0.7 and 0.6 mg/kg by day 17. In 1969, Alumot and Bielorai (1969) analyzed several grains fumigated with commercial mixture No. 5 (Table 1) and aired at different temperatures. These data were further analyzed in 1975 (Bielorai and Alumot, 1975) along with new data on carbon tetrachloride and other fumigants from both laboratory and field studies. They found more rapid desorption of fumigant residues from whole cereal grain aired at low (15°C) rather than high (30°C) temperatures, although the absorption rate of CCl_4 was less temperature dependent than those of the other fumigants. However, for ground grains the temperature effect was annulled, indicating that grain structure is the principal factor involved. Bielorai and Alumot (1975) suggest that unchanged fumigant residues are present in two forms, loosely and firmly bound, and that the loosely bound desorbs rapidly while the firmly bound desorbs more slowly and is the temperature dependent component.

Lynn and Vorches (1957) reported carbon tetrachloride residues in fumigated wheat and wheat fractions that were analyzed before and after treatment with commercial fumigant mixtures No. 2 and No. 3 (Table 1) at dosages recommended by the U.S. Dept. of Agriculture. The normal dosage for each mixture is 2 gallons/1,000 bushels. The residues found are given in Table 2 (mixture numbers referred to in the table are described in Table 1).

TABLE 2

CCl_4 Residues, parts per million (mg/kg)*

Product	Before Fumigation	Normal Dosage #2 Commercial Mixture (CCl_4-CS_2)	Normal Dosage #3 Commercial Mixture (CCl_4-EDC-EDB)
Wheat (soft)	0.5	115	(76 ppm org. Cl)
Flour	0.5	21	10
Shorts	---	39	28
Bran	---	88	43

Product	Before Fumigation	Triple Dosage #2 Commercial Mixture (CCl_4-CS_2)	Triple Dosage #3 Commercial Mixture (CCl_4-EDC-EDB)
Wheat (soft)	0.5	270	140
Flour	0.5	74	34
Shorts	---	79	72
Bran	---	67	204

*Source: Lynn and Vorches, 1957

Berck (1974) analyzed wheat, wheat fractions, and bread fractions for carbon tetrachloride residue following treatment with Dowfume EB-5® (CCl_4, EDC, EDB; 63:30:7 percent respectively, by weight) at a dosage of four imperial gallons/1,000 bushels. Samples were analyzed after different periods of aeration. The highest residues were found in the fumigated wheat; they ranged from 72.6 mg/kg (1-week aeration) to 3.2 mg/kg (7-weeks aeration). The wheat fractions had residues ranging from 0.20 to 0.93 mg/kg for flour, 0.43 to 3.53 mg/kg for bran, and 0.20 to 1.65 mg/kg for middlings. Bread made from wheat aerated for three days had residues of about 0.04 mg/kg in the upper and lower crusts and 0.13 mg/kg in the crumbs. In bread made from wheat aerated for seven weeks, the upper crusts had no residue, the lower crust had 0.2 mg/kg, and the crumbs had 0.01 mg/kg.

Results of these studies summarized by the National Research Council (1978) indicate that the amount of carbon tetrachloride residue depends on the fumigant dosage, storage conditions, length of aeration, and extent of processing. Usually, proper storage and aeration reduce CCl_4 residues to trace amounts. However, several studies indicate that, despite prolonged aeration and proper storage conditions, the residues may persist at low levels for as long as a year.

A bioconcentration factor (BCF) relates the concentration of a chemical in aquatic animals to the concentration in the water in which they live. The steady-state BCFs for a lipid-soluble compound in the tissues of various aquatic animals seem to be proportional to the percent lipid in the tissue. Thus, the per capita ingestion of a lipid-soluble chemical can be estimated from the per capita consumption of fish and shellfish, the weighted average percent lipids of consumed fish and shellfish, and a steady-state BCF for the chemical.

Data from a recent survey on fish and shellfish consumption in the United States were analyzed by SRI International (U.S. EPA, 1980). These data were used to estimate that the per capita consumption of freshwater and estuarine fish and shellfish in the United States is 6.5 g/day (Stephan, 1980). In addition, these data were used with data on the fat content of the edible portion of the same species to estimate that the weighted average percent lipids for consumed freshwater and estuarine fish and shellfish is 3.0 percent.

A measured steady-state bioconcentration factor of 30 was obtained for carbon tetrachloride using bluegills (U.S. EPA, 1978). Similar bluegills contained an average of 4.8 percent lipids (Johnson, 1980). An adjustment factor of 3.0/4.8 = 0.625 can be used to adjust the measured BCF from the 4.8 percent lipids of the bluegill to the 3.0 percent lipids that is the weighted average bioconcentration factor for carbon tetrachloride and the edible portion of all freshwater and estuarine aquatic organisms consumed by Americans is calculated to be 30 x 0.625 = 18.75.

Inhalation

Carbon tetrachloride has been measured extensively in the atmosphere; its distribution is well understood. Of all the nonfluorinated halomethanes, CCl_4 has been studied most extensively.

Historically, CCl_4 was used as an inhalation anesthetic (NIOSH, 1975). Smith (1867) reported the results of 52 cases in which carbon tetrachloride was the anesthetic agent. He concluded that it was useful in removing pain while producing no nausea or sickness (NIOSH, 1975).

The occurrence of CCl_4 in the atmosphere is due largely to its volatility. A number of researchers have measured the amounts of CCl_4 in the atmosphere. Their results are given in Table 3. It is evident that there are no major gradients in the atmospheric distribution of CCl_4 between the continental and marine air masses. Comparison of the amounts of CCl_4 in the Southern Hemisphere reveals a slightly lower concentration than that found in the Northern Hemisphere (NRC, 1978); however, the gradient is decreasing as emissions in the more industrialized Northern Hemisphere have stabilized with the new era of environmental awareness. Thus, the global atmospheric distribution is approaching homogeneity. Some extremely high CCl_4 concentrations have been reported in urban air. An average annual amount of 0.0091 mg/m^3 was found in Tokyo between April 1974 and April 1975 (Ohta, et al. 1976). This was the highest level ever measured over an extended period of time; the data have not been confirmed (NRC, 1978). The maximum quantity measured in the atmosphere was detected by Lillian, et al. (1975); they found 0.117 mg/m^3 in Bayonne, New Jersey. Su and Goldberg (1976) detected a high CCl_4 concentration in Grenoble, France: 0.0098 mg/m^3.

CCl_4 is primarily of anthropogenic orgin (Altshuller, 1976; Lovelock, et al. 1974; Wilkniss, et al. 1973; Singh, et al. 1976). Of all of the halocarbons, it is the most widely distributed (NRC, 1978).

TABLE 3

Summary of Atmospheric Concentrations of Carbon Tetrachloride (mg/m^3)

Compound	Continental Background	Marine Background	Urban Range
Carbon	.00013 ± .000065[a]		
Tetrachloride	.00079 ± .000085[b]	.000083 ± .000026[e]	.00087 ± .00013[d]
(CCl_4)	.00086 ± .000065[c]	.00083 ± .00010[d]	.00078 ± .117[g]
	.00075 ± .000052[d]	.00072 ± .00007[f]	.0091[h]
	.00078 ± .000098[a]		.00078 ± .00975[i]

a. Grimsrud and Rasmussen, 1975
b. Cronn, et al. 1976
c. Pierotti and Rasmussen, 1976
d. Singh, et al. 1976
e. Pierotti, et al. 1976
f. Cox, et al. 1976
g. Lillian, et al. 1975
h. Ohta, et al. 1976
i. Su and Goldberg, 1976

Dermal

It has long been known that chlorinated aliphatic hydrocarbon solvents can penetrate animal or human skin (Stewart and Dodd, 1964). Absorption of these solvents through animal skin has been investigated in the laboratory, but there have been few controlled studies of human exposure.

Historically, CCl_4 was used as a waterless shampoo (NIOSH, 1975). In 1909 recommendations were made to label the compound as a poison after its use in this capacity resulted in death. It continued to be used in some European countries as a hair shampoo despite further reports of serious illness and death (NIOSH, 1975).

Early in the 20th century, health hazards were being reported from industrial uses of CCl_4. In Germany, Lehmann confirmed evidence of "unwelcome effects" when CCl_4, used as a cleaning agent, was brushed on by hand (NIOSH, 1975). In 1915, Hamilton reported that men working with CCl_4 without the protection of gloves developed dermatitis on their hands and arms (NIOSH, 1975).

Information was not found on levels or frequencies of dermal exposures. Carbon tetrachloride is no longer on the market as a hair shampoo, and through the practice of strict safety regulations, dermal exposure in the work environment has been minimal.

PHARMACOKINETICS

Much of this section is extracted from reviews compiled by NIOSH (1975) and von Oettingen (1964).

Absorption

Carbon tetrachloride is readily absorbed through the lungs and more slowly through the gastrointestinal tract (Nielsen and Larson, 1965). It can also enter the body by penetration through the skin. The rate and amount of absorption are enhanced with the ingestion of fat (Nielsen and Larson, 1965) and alcohol (Nielsen and Larson, 1965; Folland, et al. 1976; Moon, 1950). Nielsen and Larson (1965) found high CCl_4 concentrations in animal testicular fatty tissues, liver, brain, bone marrow, and kidneys.

Lehmann and Hasegawa (1910) showed that the rate of absorption through the lungs decreases with the duration of exposure. Von Oettingen, et al. (1949, 1950) followed the absorption of CCl_4 through the lungs by determining its level in the blood at different intervals. The results are given in Table 4.

These findings confirm the observation of Lehmann and Hasegawa (1910) that the rate of absorption decreases gradually and finally reaches an equilibrium. Furthermore, this equilibrium is established earlier with high concentrations because the vascular collapse and depression of respiration will later interfere with the absorption. Lazarew (1929) suggested that the absorption of CCl_4 is limited because of its moderate solubility in water. Powell (1945) determined that the distribution coefficient (concentration in the liquid phase divided by that in the gaseous phase) between CCl_4 dissolved in water and that present in air is 1.04 at 20°C and 0.46 at 37°C. The concentration in air varies between 0.33 and 3.44 mg/100 ml, corresponding to 500 and 5,000 ppm (mg/kg). He showed that the distribution coefficient between CCl_4 dissolved in blood and that present in air is constant for a given specimen throughout the above range of concentrations and varies with different specimens between 3.6 and 5.2 at 20°C and between 1.8 and

TABLE 4

Concentration of Carbon Tetrachloride in the Blood of Dogs (mg/l)
During Exposure to Concentrations of 15,000 and 20,000 mg/l CCl_4 in Air
(Averages of 5 Experiments)*

TIME (in minutes)

10	30	60	90	120	150	180	210	240	300	360	420	480	Later
A. CONCENTRATION = 15,000 mg/l													
13.33	19.22	23.08	27.05	29.53	27.57	27.23	28.78	29.92	34.17	32.09	36.23	35.64	35.26
B. CONCENTRATION = 20,000 mg/l													
17.73	18.94	29.55	33.17	33.39	35.40	36.49	-	36.20	38.10	-	-	-	38.5

*Source: von Oettingen, et al. 1949

2.5 at 37°C. These findings support the observation that changes in the CCl_4 level in the blood are due to physiological functional changes.

An investigation of the absorption of CCl_4 from the gastrointestinal tract of dogs was performed by Robbins (1929). He found that considerable quantities are absorbed from the small intestine, lower quantities from the colon, and still lower quantities from the stomach. From an observation by Lamson, et al. (1923) it appears that the absorption from the gastrointestinal tract may vary with different species because it occurs more rapidly in rabbits than in dogs.

The amount of CCl_4 absorbed through the skin does not appear to be significant when compared to the other routes of entry. Lapidus (1929) studied CCl_4 in the blood, liver, and brain of the rabbit. Four animals were used in this investigation, which entailed immersion of one ear of each rabbit into CCl_4. Ear immersion times were 5, 6, 8, and 9 hours; precautions were taken to avoid inhalation of CCl_4 vapor. Following exposure, analysis for CCl_4 showed that the blood contained 0.12 to 0.13 mg/g; the liver, 0 to 90 mg/g; and fat, 0 to 300 mg/g. A trace of CCl_4 was found in the brain of the rabbit whose ear was immersed for 9 hours, but none was detected in the brains of the remaining three rabbits. The lowest detectable limit of the analytical technique used for the brain analysis was 5 mg.

Contrary to this finding, Kionka (1931) claimed that CCl_4 is not absorbed through intact or scarified skin but is absorbed quite readily through burnt (denuded) skin. However, McCord (1932) administered CCl_4 under a leak-proof bandage placed on the clipped abdominal skin of three animals (species not specified). He injected either 7.5, 1.6, or 1.2 mg/kg CCl_4 under the bandages three times per day for 7 or 8 days. The animal receiving 7.5 mg/kg CCl_4 died on the seventh day. The animal receiving the 1.6 mg/kg injection died on the eighth day. The remaining animal, receiving 1.2 mg/kg CCl_4, was killed on the seventh day. Autopsies were performed but were limited to macroscopic observations. Subcutaneous necrosis was evident where the CCl_4 had been applied; the livers showed a dark purple mottling, and inflammation was seen throughout the body of each animal. This study suggests that CCl_4 is absorbed through animal skin.

Lande and Dervillee (1936) studied Kionka's hypothesis in rats and guinea pigs. They applied CCl_4 to normal, scarified, and burnt skin and also to skin with open lesions. No evidence was found that it was absorbed through intact or scarified skin even when application continued for 6 to 8 hours. When applied to open lesions or burnt areas, sufficient quantities were absorbed so as to result in liver and kidney injury. Tabusso (1941) produced chronic CCl_4 poisoning in rabbits by daily application of a 5 percent solution in ether to the shaved skin. Beamer, et al. (1950) studied in monkeys the cutaneous absorption of CCl_4 vapors containing radioactive carbon. They found that after a 270-minute exposure to concentrations of 1,150 ppm (mg/l), the radioactivity of the blood was equivalent to 0.30 mg CCl_4/l and that the expired air contained 0.003 mg/l. These experiments show that carbon tetrachloride vapors will penetrate through the intact skin of animals in very small amounts.

Larger amounts may be absorbed through the human skin (von Oettingen, 1964).

Stewart and Dodd (1964) investigated the absorption of CCl_4 through human skin. Three subjects each immersed one thumb in CCl_4 for 30 minutes. The concentrations of CCl_4 in the exhaled air were measured at 10, 20, and 30 minutes of immersion. Ranges of CCl_4 concentrations in the exhaled air measured at these time periods were 0.0250 to 0.819 µg/l, 0.25 to 3.27 µg/l, and 0.69 to 5.23 µg/l, respectively. Five hours later, CCl_4 was still measurable in the exhaled air of these subjects. The investigators concluded that the amount of CCl_4 that could penetrate the skin depends on the type of skin, the area exposed, and the duration of exposure. Using the data from the experimental exposure of one thumb, they estimated that the amount of CCl_4 absorbed during topical exposure of both hands for 30 minutes would be equivalent to a vapor exposure of about 10 mg/l for 3 hours.

Distribution

Robbins (1929) studied the distribution of CCl_4 in dogs after oral administration. He found the highest concentration in the bone marrow. The amount found in the liver, pancreas, and spleen was one-fifth of the amount found in the bone marrow. Von Oettingen, et al. (1949, 1950) determined the concentration of CCl_4 in various organs of dogs exposed to 97,500 and 130,000 mg/m^3 CCl_4 in air. The results are summarized in Table 5. These findings indicate that with inhalation, the concentration in the brain is higher than that in the heart, liver, and blood. Findings further

TABLE 5

Concentration of Carbon Tetrachloride in Various Organs of Dogs Exposed to CCl_4 in Air*

Dog Number	Exposure Time (Minutes)	Concentration (mg/100 ml)			
		Heart	Liver	Brain	Blood
		A. 97,500 mg/m^3.			
20	640	56.80	43.35	71.40	44.15
21	485	73.30[a]	31.63	62.03	31.16
22	505	40.10	31.63	48.05	32.40
23	495	48.05	46.46	74.20	29.75
24	250	49.62	25.06	67.90	30.58
AVERAGE	475	53.57 (48.64)[b]	35.63	64.72	33.61
		B. 130,000 mg/m^3.			
29	15	25.53	9.85	29.55	19.19
27	30	33.00	25.83	44.62	21.50
25	45	19.30	17.50	32.56	10.45
28	335	69.80	60.66	84.80	43.20
26	345	85.09	57.63	82.60	33.80
AVERAGE	154	46.54	34.29	54.83	25.63
AVERAGE OF DOGS 28 AND 26	340	77.44	59.14	83.70	38.50

*Source: von Oettingen, et al. 1949

[a] Heart showed fatty degeneration.

[b] Average value excluding No. 21.

indicate that the concentration in the brain increases with time of exposure and the concentration of CCl_4 in the air.

McCollister, et al. (1950) studied the organ distribution in monkeys after inhalation of radioactive CCl_4 in concentrations of 299 mg/m^3 for 139 and 334 minutes. They reported the following ratios:

Blood	1.00
Depot fat	7.86
Liver	3.00
Bone marrow	2.97
Bone, lung, muscle, spleen, heart, kidney & brain	0.14-0.96

McConnell, et al. (1975) found CCl_4 in human tissues as follows (µg/kg, wet tissue):

Kidney	1-3
Liver	1-5
Body fat	1-13.6

Recknagel and Litteria (1960) worked with rats and demonstrated that after oral administration, the liver concentration of CCl_4 increases for 1.5 hours and then continuously decreases. It appears that the organ distribution of CCl_4 varies with the route of administration, its concentration, and the duration of exposure. On the cellular level, McLean, et al. (1965) found CCl_4 in all cell fractions with higher concentrations in ribosomes.

Metabolism

When CCl_4 is administered to mammals, it is metabolized to a small extent (most is excreted through the lungs). The metabolites include chloroform, hexachloroethane, and carbon dioxide (CO_2). Research efforts have revealed that these metabolites play an important role in the overall toxicity of CCl_4. Some of the CCl_4

metabolic products are incorporated into fatty acids by the liver and into liver microsomal proteins and lipids (Gordis, 1969).

The chemical pathology of liver injury induced by CCl_4 is generally viewed as an example of lethal cleavage (Recknagel and Glende, 1973). The initial event is thought to involve the homolytic cleavage of a C-Cl bond of CCl_4, thus liberating trichloromethyl and chlorine free radicals. There are two major views on the consequences of this cleavage; both views consider the high reactivity of the free radical products of the cleavage.

The first possibility is direct attack (via alkylation) by toxic free radical metabolites of CCl_4 metabolism on cellular constituents, especially protein sulfhydryl groups (Butler, 1961). In homolytic fission, the two odd-electron fragments formed would be trichloromethyl and monatomic chlorine free radicals (e.g., $CCl_4 \longrightarrow {}^{\cdot}CCl_3 + Cl^{\cdot}$). Verifying this hypothesis, Fowler (1969) detected hexachloroethane (CCl_3CCl_3) in tissues of rabbits following CCl_4 intoxication.

The alternative view has emphasized peroxidative decomposition of lipids of the endoplasmic reticulum as a key link between the initial bond cleavage and pathological phenomena characteristic of CCl_4 liver injury (Tracey and Sherlock, 1968). Thus, CCl_4 binds to cytochrome P-450 apoprotein and is cleaved at the locus to yield extremely short-lived free radicals which initiate peroxidative decomposition of polyenoic lipids (Fishbein, 1976). The autocatalytic decomposition of the lipid spreads from the initial locus and lipid peroxides, and hydroperoxides probably also move to more distant sites where they decompose to yield new free radicals

(Fishbein, 1976). Rapid breakdown of structure and function of the endoplasmic reticulum is due to decomposition of the lipid and to attack on protein functional groups, especially sulfhydryl groups, by lipid peroxides (Recknagel and Glende, 1973).

This intracellular process of lipid peroxidation has been linked to adverse effects. Recknagel, et al. (1973) explain that peroxidative decomposition of the membrane structural lipids disrupts normal structure and function, usually catastrophically. For example, peroxidative decomposition of red cell lipids has been correlated with an increase in red cell permeability and hemolysis in vitamin E deficiency.

It has been hypothesized that CCl_4 is the initiator of some process which proceeds autocatalytically. A study in which rats were treated with a variety of drugs has supplied further support to this hypothesis. For example, administration of phenobarbital for several days increases the available activity of the liver microsomal drug-metabolizing enzyme system. Rats treated with such a drug show an enhanced susceptibility to a fixed dose of CCl_4, and the double bond shift in the microsomal lipids of such rats in response to a fixed dose of CCl_4 is markedly enhanced. When a drug which inhibits microsomal electron flow at the cytochrome P-450 level is administered, the toxic effect of CCl_4 is ameliorated (Recknagel, et al. 1973). Studies with rats reveal that pretreatment with phenobarbital to stimulate cytochrome P-450 increased metabolism of orally administered CCl_4 to CO_2, markedly increased fat in the liver, and increased plasma concentrations of bilirubin (McLean and McLean, 1966; Garner and McLean, 1969).

Pre-exposure to DDT has also been shown to increase both the P-450 content of rat liver cells and subsequently the toxicity of CCl_4 (McLean and McLean, 1966; McLean, 1971). Ethanol administration increased the activity of the liver hydroxylating enzyme system. Ethanol pretreatment increased the necrotic liver injury due to CCl_4 but had less effect on the liver fat accumulation caused by CCl_4 (Wei, et al. 1971).

Litterst, et al. (1973) found that dogs chronically treated with phenobarbital for 12 months and then given a single dose of CCl_4 were affected to a greater extent than dogs given only CCl_4, as indicated by increased liver triglyceride content, increased diene conjugates, and increased serum glutamic-oxalacetic transaminase (SGOT).

Klaassen and Plaa (1969) found that CCl_4 promotes lipid peroxidation in the liver of rats at oral doses of 0.3 to 1.0 ml/kg, but not at doses of 0.1 ml/kg. Hashimoto, et al. (1968) compared lipid peroxidation in the liver of a woman who died with massive liver necrosis after drinking CCl_4 with that of a victim who died from a traffic accident. They concluded, on the basis of liver lipid conjugated dienes, that extensive peroxidative degeneration occurred as a result of the CCl_4 poisoning.

Investigations have been made of the mechanism of action of CCl_4. Nakata and Higaki (1969) summarized the action of CCl_4 on an isolated perfused rat liver:

1. Temperature increase in hepatic blood flow in the early stages and marked decrease in the advanced stages following the toxic injury of the liver cell.

2. Impairment of bile production.
3. Extremely low oxygen saturation of the hepatic vein blood flow, apparently the result of deterioration of oxygen uptake by the liver cells injured by CCl_4.
4. Histological lesions comprising:
    - a. Eosinophilic degeneration and decrease in amount of glycogen in the liver cell at the inital stage of intoxication.
    - b. Marked swelling of the liver cell accompanied by marked stenosis of the sinusoid at the middle zone of the liver lobule.
    - c. Necrosis of the liver cell at the central zone of the liver lobule.
    - d. Proliferation of macrophages which obliterates the sinusoidal lumen in the central zone of the liver lobule at the advanced stage of CCl_4 intoxication.

Excretion

Lehmann and Hasegawa (1910) stated that 78.7 percent of the amount of inhaled CCl_4 is excreted through the lungs within six hours after exposure. McCollister, et al. (1950) found that approximately 50 percent of absorbed radioactive CCl_4 is eliminated through the lungs. The remaining 50 percent is eliminated in some form in the urine and feces. According to Robbins (1929), it is not excreted, as such, in the urine by dogs. This finding was confirmed by Barrett, et al. (1939). All investigators agree that the largest portion of the absorbed CCl_4 is rapidly excreted. Beamer, et al.

(1950) reported that following inhalation of labeled CCl_4, no radioactive CCl_4 was detectable in the blood after 48 hours, nor was it detectable in the expired air after 6 days.

A study on "Drinking Water and Health" (NRC, 1977) concurs that CCl_4 is primarily excreted through the lungs in both animals and humans. A summary of this study's findings reveals that the excretion products are 85 percent as the parent compound, 10 percent carbon dioxide, and smaller quantities of other products including chloroform.

EFFECTS

Acute, Subacute, and Chronic Toxicity

Norwood, et al. (1950) reported the occurrence of 2 fatalities, 1 near fatality, 4 poisonings requiring hospitalization, and 51 mild industrial poisonings in two communities over a period of 1 year. Smyth (1935) noted 28 fatalities, 14 of which resulted from the ingestion of CCl_4; 120 acute and subacute poisonings; and 7 cases of chronic poisoning. Subsequently, 28 poisonings resulting from CCl_4 ingestion (including 10 fatalities) and 202 cases from inhalation (including 29 fatalities) have been reported. The actual incidence of such poisonings is doubtless much greater because many poisonings are not attributed to CCl_4 and others are not published in the medical literature (von Oettingen, 1964).

Many poisonings have resulted from the accidental or suicidal ingestion of CCl_4 or from its medicinal use as an anthelmintic. The vast majority, however, have resulted from the inhalation of its vapors when used as a solvent or dry cleaning agent (von Oettingen, 1964). Still other poisonings have been the result of dermal exposures through the use of CCl_4 in shampoos (NIOSH, 1975). Finally, some have resulted from its use in fire extinguishers (Dudley, 1935).

Direct application of CCl_4 onto the human skin causes a burning and stinging sensation within 5 minutes (Oettel, 1936). The maximum pain is reached 6 minutes later and is associated with erythema, hyperemia, and wheal formation, later followed by vesication (Oettel, 1936).

Hall (1921a,b) demonstrated the effectiveness of CCl_4 as a vermicidal agent in treatment of hookworm infestations. The usage of CCl_4 in such capacity stimulated considerable research efforts to investigate the pharmacologic and physiologic effects of CCl_4 on humans (NIOSH, 1975). The effects of oral doses of CCl_4 as a human anthelmintic, administered to condemned prisoners in Ceylon has been reported (Docherty and Burgess, 1922; Docherty and Nicholls, 1923). Three of the prisoners received 4 ml CCl_4, two received 5 ml CCl_4, and one received 5 ml plus an additional 3 ml two weeks after the first dose. Execution of the prisoners occurred 3 to 15 days after the CCl_4 administration. Autopsies were performed and the findings varied. The livers of some showed no major microscopic or macroscopic changes whereas the livers of others showed marked fatty degeneration. From such data, a dose-response relationship would be difficult to determine (NIOSH, 1975).

The therapeutic dose recommended for adults was 2 to 3 ml in capsule form and 0.13 ml/year for infants and children up to 15 years of age (von Oettingen, 1964). As emphasized by von Oettingen (1964), such doses, which are followed by doses of Epsom salts, have caused toxic effects only exceptionally. Horrocks (1934) reported one fatality from its medicinal use.

However, oral poisonings have occurred to a great extent, as reported by a number of authors (Docherty and Burgess, 1922; Beattie, et al. 1944; NIOSH, 1975; Kirkpatrick and Sutherland, 1956; Dawborn, et al. 1961). A paraphrased summary of the symptoms of such oral poisoning is given below (von Oettingen, 1964). Following ingestion of CCl_4, the patient experiences a burning sensation in the mouth, esophagus, and stomach. Depending upon the dose, this is sooner or later complicated by abdominal pain, nausea, and vomiting. Some patients develop hiccoughs. The tongue is coated. These symptoms are soon followed by diarrhea, which later may be followed by constipation and occasionally by gastric and intestinal hemorrhages which, in rare cases, may also be seen in the mouth and pharynx. Again, depending upon the dose along with other factors, the patient becomes jaundiced, the liver becomes enlarged and tender, and this may be associated with ascites and generalized edema. Soon after the ingestion, the patient feels dizzy, may suffer from headache and become confused, semiconscious, and delirious. The patient may become restless and develop choreatic movements. Finally, consciousness is lost and the patient passes into coma. Some patients complain of visual disturbances and edema of the eyelids and develop hemorrhages of the sclerae. In severe cases, circulatory disturbances may develop, characterized by lowered or increased blood pressure, thin and rapid pulse, and signs of congestive heart failure with cyanosis. Nakata and Higaki

(1969) demonstrated these types of changes in vitro, through experiments with the rat liver.

Conway and Hoven (1946) point out that certain electrocardiographic changes may be observed indicating degenerative processes in the heart muscle, such as sinus bradycardia, followed by auriculo-ventricular rhythm, auricular fibrillation, and sinus arrhythmias. The respiration varies with the condition of the patient. If he is in collapse, it will be rapid and shallow; if he is comatose, it may be labored and dyspneic, and pulmonary edema and hemorrhages may develop. Eventually, disturbances develop characterized by polyuria and followed by oliguria which may pass into anuria. The urine of such patients is rich in albumin and may contain blood and casts. If the liver is damaged, the urine will contain urobilinogen, urobilin, and bile pigments. The nonprotein nitrogen level in the blood will be increased and the patient may suffer from hypoprothrombinemia, hypochloremia, and signs of acidosis. Death may ensue after 8 hours, or 3, 5, or 10 days, and sometimes later.

Postmortem reports on pathological changes in patients after the ingestion of CCl_4 are not numerous. McMahon and Weiss (1929) examined a 34-year-old male alcoholic who died five days after drinking one ounce of CCl_4. They discovered some reddish-brown fluid in the abdominal cavity, early atheromatous lesions in the heart, congested and edematous lungs with scattered petechial hemorrhages, enlarged and congested kidneys, marked erosion of the esophagus, and a congested and enlarged fatty liver.

Acute toxicity of CCl_4 by inhalation for humans has been reported by different investigators (Davis, 1934; Stewart, et al.

1961; Smith, 1950; NIOSH, 1975; von Oettingen, 1964). Inhalation studies have also been performed on animals (Adams, et al. 1952; Prendergast, et al. 1967; Wong and DiStefano, 1966). The human studies indicate that with single exposure to low concentrations, there is considerable variation in symptoms among different persons and that the acute toxicity is relatively low in contrast to that with repeated exposure. Cases in which exposure is light may be restricted to such symptoms as moderate irritation of the eyes, moderate dizziness, and headache, which disappear promptly upon discontinuation of the exposure.

The immediate effects from acute inhalation exposure to higher concentrations of CCl_4 consist of the same symptoms as described above, but in addition the patient may become nauseated and suffer from loss of appetite, mental confusion, agitation, and the feeling of suffocation. In severe cases, the patient may lose consciousness and develop fever and chills. The tongue may be furred and the patient may suffer from vomiting with bloody or bile-stained vomitus which may last for days, colicky pain, and diarrhea with liquid brown-black or bloody stools (von Oettingen, 1964). This tendency for hemorrhages may also result in bleeding from the gums and nose, hemorrhages under the skin, and macular papular rashes. The colicky pain may be associated with a marked abdominal resistance simulating the "acute abdomen" and thus has been mistaken for appendicitis and peptic ulcer. Following such an acute episode, the patient feels tired and weak and frequently suffers from headache. The patient may develop muscular twitchings and epileptic convulsions. In a few instances, paralysis (hemiplegia), and polyneuritis have been reported (von Oettingen, 1964).

In more severe inhalation poisoning blood pressure may be lowered, but as renal complications develop, the blood pressure is usually elevated and the cardiac output decreases because of increased peripheral resistance. The pulse may be accelerated. In the case of severe inhalation poisoning, the patient may collapse. Electrocardiograms have shown changes characteristic of myocardial injury characterized by sinus bradycardia and followed by auriculoventricular rhythm, auricular fibrillation, and sinus arrhythmias (von Oettingen, 1964).

Depending upon the condition of the patient, respiration may be normal, rapid and shallow, or slow and labored. The latter is evident especially if circulatory failure is imminent and pulmonary edema develops. Thompson (1946) found that early roentgenograms of the lungs may show pulmonary involvement.

In most instances after the severe inhalation exposure, the patient develops signs of liver injury within a few days. The patient becomes jaundiced and the liver becomes enlarged and tender. This is toxic hepatitis, which may pass into yellow atrophy and, in more protracted cases, eventually into cirrhosis of the liver. In the early stages of liver injury, even before a marked enlargement occurs and while liver function tests such as the cephalin-flocculation test are still normal, the level of serum glutamic-oxalacetic transaminase (SGOT) may be markedly elevated (von Oettingen, 1964).

As signs of liver injury develop, and sometimes in their absence, injury of the kidneys may dominate the clinical picture and be responsible for early death (von Oettingen, 1964). Kittleson and Borden (1956) characterized renal failure by three phases. The first phase is characterized by polyuria and nocturia, which may result in severe dehydration, followed by oliguria and finally by diuresis. The renal injury may result in acute nephritis with albumin, red and white cells, and casts in the urine. In some patients, the presence of acetone and sugar in the urine has been reported. The oliguria may be associated with increased blood levels of potassium, indican, phenol, cresol, creatinine, and urea; the latter may result in uremia. In other instances, the injury may consist in necrotizing nephrosis with comparatively little changes in the urinary composition. The renal blood flow and glomerular filtration rate are decreased, and the former seems to be mainly responsible for the maintenance of oliguria, being the sequela rather than the cause of renal failure (von Oettingen, 1964). During the early stage of oliguria, abnormal tubular back diffusion of the filtrate may play an important role. Oliguria may develop as early as 24 hours or 3 to 4 days after onset of the poisoning and may persist for 12 to 14 days and even longer (von Oettingen, 1964).

In the early stages after severe inhalation poisoning and during the period of polyuria, the blood may show some polycythemia, but later this may be followed by anemia and lowering of the hematocrit levels because of hemodilution. The most important changes in the blood are, however, related to the biochemical composition of the blood which reflects the renal and hepatic injury. As soon as the renal injury develops, the nonprotein nitrogen and urea-nitrogen levels in the blood are increased and may reach extremely high values. The creatinine, indican, phenol, and cresol levels may also be increased. In the case of liver injury, as related to the blood, the icteric index is usually increased, and the levels of sugar and phospholipids, along with the ratio of cholesterol esters over cholesterol, are reduced. The prothrombin time and the fibrinogen content may be reduced, resulting in an increased clotting time. The chloride level is frequently lowered by hemodilution or severe vomiting, and the potassium level may be elevated. This increase in potassium may contribute to ventricular fibrillation or cardiac arrest (von Oettingen, 1964).

Carbon tetrachloride poisoning can also result in blurred and double vision. Constriction of the visual field and toxic amblyopia have been reported (NIOSH, 1975; von Oettingen, 1964). Conjunctival hemorrhages are common. Retinal hemorrhages and exceptional cases of the degeneration of the optic nerve have been reported (von Oettingen, 1964).

Two cases involving the pancreas following inhalation exposure to CCl_4 were reported by Jahnke (1953). Both patients became listless and developed hepatic and circulatory disturbances and sensitivity of the pancreas to pressure. Such disturbances were long-term and had not completely subsided after 10 months.

Chronic inhalation poisoning is the result of continued low exposures. Cases of such occurrences have been reported by Butsch (1932), Wirtschafter (1933), Strauss (1954), von Oettingen (1964), and others. The clinical picture of chronic CCl_4 poisoning is much less characteristic than that of acute poisoning. Von Oettingen (1964) has reviewed the symptoms. Patients suffering from this condition may complain of fatigue, lassitude, giddiness, anxiety, and headache. They suffer from paresthesias and muscular twitchings and show increased reflex excitability. They may be moderately jaundiced, have a tendency to hypoglycemia, and biopsy specimens of the liver may show fatty infiltration. Patients may complain of lack of appetite, nausea, and occasionally of diarrhea. In some instances, the blood pressure is lowered which is accompanied by pain in the cardiac region and mild anemia. Other patients develop pain in the kidney region, dysuria, and slight nocturia and have urine containing small amounts of albumin and a few red blood cells. Burning of the eyes and, in a few instances, blurred vision are frequent complaints of those exposed. If these symptoms are not pronounced or of long standing, recovery usually takes place upon discontinuation of the exposure if the proper treatment is received (von Oettingen, 1964).

Postmortem reports on pathological changes in patients after inhalation of CCl_4 are generally limited to findings in the liver and kidneys. The liver may show nutmeg appearance and fatty degeneration even in the absence of clinical signs and symptoms of liver injury. In other instances, centrilobular necrosis and hemorrhages with infiltration of leukocytes and histiocytes and collapse of the lobules with condensation of the reticular framework within these areas are seen. After chronic exposure, there may be evidence of regeneration of the liver cells (von Oettingen, 1964).

Postmortem changes in the kidney are characterized by nephrosis, by a distention of Bowman's capsule with albuminous precipitates, and by swelling of the lining cells. The cells of the convoluted tubules may be swollen and vacuolated; later, degenerative changes may be seen in Henle's loops, associated with granular, hyaline, and cellular casts in the tubules. After chronic exposure, regenerative changes may be visible in these regions. In other cases, the kidneys may offer the picture of acute hemorrhagic nephritis (von Oettingen, 1964).

Other postmortem organ changes are less characteristic for CCl_4 poisoning and vary considerably with the clinical picture. Some changes may occur that are a direct result of the changes occurring in the primary target organs of CCl_4. Stasis of various organs is the most outstanding feature of cardiac failure. The brain and lungs may be edematous. The intestines may be hyperemic and covered with numerous petechial hemorrhages, and the spleen may be enlarged and hyperemic. Occasionally the adrenal glands may show degenerative changes of the cortex, and the heart may undergo toxic myocarditis (von Oettingen, 1964).

Synergism and/or Antagonism

A description of the entire clinical picture of the toxicity of CCl_4 should consider the role played by alcohol in the genesis of severe CCl_4 poisoning (von Oettingen, 1964). A number of researchers have reported on this phenomenon (Stevens and Forster, 1953; Kirkpatrick and Sutherland, 1956; Joron, et al. 1957; New, et al. 1962; Traiger and Plaa, 1971). It has been established that habitual ingestion of alcoholic beverages and also occasional use may increase the dangers from comparatively moderate exposure. This fact is illustrated by reports on simultaneous exposure of abstinent persons and consumers of alcohol to the same concentration with only the latter becoming seriously ill (von Oettingen, 1964).

Traiger and Plaa (1971) investigated the differences in the potentiation of CCl_4 by pretreatment with methanol, ethanol, and isopropanol in rats. The activity of serum glutamic-pyruvic transaminase (SGPT) was monitored to assess the effects of hepatotoxicity. Methanol, ethanol, and isopropanol displayed potentiating ability and produced elevated activity of SGPT. The most marked potentiation was produced by isopropanol. The administration of the alcohols or CCl_4 alone did not change the levels of SGPT.

Wei, et al. (1971) investigated the potentiation of CCl_4 hepatotoxicity by ethanol and cold. This was accomplished by pretreating rats with ethanol and exposing rats to a cold temperature (18 hours at 4^oC). Indices of hepatotoxicity were SGPT levels and liver triglyceride levels. In both male and female rats, the SGPT levels increased after both ethanol and cold exposures in response to the CCl_4. The authors postulate that the ethanol releases norepinephrine, which increases the susceptibility of the liver to CCl_4. According to Davis (1934), very obese or undernourished persons suffering from pulmonary diseases or gastric ulcers or having a tendency to vomiting, liver or kidney diseases, diabetes, or glandular disturbances are especially sensitive to the toxic effect of CCl_4 (von Oettingen, 1964).

As to the antagonistic compounds associated with CCl_4, Hafeman and Hoekstra (1977) report a protective effect of dietary vitamin E, selenium, and methionine against lipid peroxidation induced by CCl_4. They monitored lipid peroxidation by the evolution of ethane, an autoxidation product of ω-3-unsaturated fatty acids. The authors concluded that the toxicity of CCl_4 decreased in correlation with ethane evolution. Thus, methionine and selenium protected against CCl_4-induced lipid peroxidation, probably by maintaining intracellular glutathione and glutathione peroxidase. Vitamin E also exhibited this protection. The authors also found that substituting cod liver oil (which is rich in ω-3-unsaturated fat) for lard in the basal diet increased CCl_4-induced ethane evolution by a factor of six.

Teratogenicity

Data concerning the teratogenicity of CCl_4 are scarce. Schwetz, et al. (1974) administered CCl_4 to Sprague-Dawley rats at 300 or 1,000 mg/l for 7 hours per day on days 6 to 15 of gestation. Results indicated that CCl_4 was not highly embryotoxic but that it does cause some degree of retarded fetal development such as delayed ossification of sternebra. Maternal toxicity was found.

Bhattacharyya (1965) studied fetal and neonatal responses to hepatotoxic agents. He found that subcutaneous injection of CCl_4 into pregnant rats and subcutaneous and intra-amniotic injection into fetuses only occasionally give rise to changes in fetal liver. When changes do occur, they vary from sinusoidal dilation and congestion or well-marked variability of staining of liver lobules to occasional centrilobular or (rarely) massive necrosis.

Mutagenicity

Very little information was found on the mutagenicity of CCl_4. Kraemer, et al. (1974) found that CCl_4 is not mutagenic in the Salmonella typhimurium or Escherichia coli reversion tests.

Carcinogenicity

In a number of studies, CCl_4 has been shown to be carcinogenic in animals, the target organ being the liver. Some of these studies will be reviewed in this section.

Rueber and Glover (1967) studied cholangiofibrosis of the liver in male and female Buffalo strain rats of varying ages. Some of the rats were given CCl_4 subcutaneously, while others were fed 3-methylcholanthrene (MCA) in the diet. Cholangiofibrosis is a lesion made up of ducts lined by irregular epithelial cells and surrounded by connective tissue. The lesion is a precursor of cholangiocarcinomas of the liver (Rueber and Glover, 1967).

Of the female rats injected with CCl_4, 4/11 52-week-old rats (36 percent) had cholangiofibrosis. The lesion was present in 11/12 8-week-old male rats (92.5 percent), 6/11 12-week-old male rats (55 percent), all of the 24- and 52-week-old male rats (100 percent), and 13/14 76-week-old male rats (93 percent). Cholangiofibrosis was less developed in the younger rats and most advanced in the oldest rats. There were atypical cells in the 24-week-old male and 52-week-old female rats. The lesions were larger in the 52-week-old rats.

Cholangiofibrosis was increased in 5-week-old male rats and 24-week-old females (50 percent) given both CCl_4 and MCA.

Cholangiofibrosis was decreased in male rats 8 to 76 weeks of age receiving both chemicals.

The cholangiofibrosis in male rats given only CCl_4 was often found in livers with severe cirrhosis. Male rats receiving CCl_4 and MCA with severe cirrhosis developed less cholangiofibrosis. In female rats given CCl_4, cholangiofibrosis was not related to the severity of the cirrhosis. (Results are given in Table 6.)

In summary, cholangiofibrosis of the liver developed in male and female rats receiving injections of carbon tetrachloride. The lesion was present in male rats of all ages, except those four weeks of age. The lesion was increased in male rats five weeks of age given both CCl_4 and MCA, whereas it was decreased in rats of all other ages. Most female rats given both chemicals also had cholangiofibrosis.

Rueber and Glover (1967) also investigated hyperplastic and neoplastic lesions of the liver. Inbred Buffalo male and female rats 4, 12, 24, 52, and 76 weeks old were given subcutaneous injections of carbon tetrachloride (CCl_4) twice a week for 12 weeks. There were 10 to 14 rats of each sex and age. Rats were given 1.3 ml/kg of body weight of a 50 percent solution of CCl_4 and corn oil. Control rats, six per group, were injected with the same amount of corn oil.

Rats survived for the 12 weeks of the study. During this period, the 52-week-old rats lost an average of 15 to 30 g, the 24-week-old rats maintained their weight, and the 12-week-old rats each gained from 20 to 30 g. The 4-week-old females weighed three

TABLE 6

Cholangiofibrosis in Male and Female Rats Given Subcutaneous Carbon Tetrachloride and Methylcholanthrene*

Age (Weeks)	Males		Females	
	CCl_4	CCl_4 and MCA	CCl_4	CCl_4 and MCA
4-5	0/14 (0%)	8/17 (47%)	0/11 (0%)	0/18 (0%)
8	11/12 (92%)	1/15 (7%)	1/12 (8%)	1/16 (6%)
12	6/11 (55%)	1/16 (6%)	1/11 (9%)	0/14 (0%)
24	11/11 (100%)	2/16 (13%)	0/10 (0%)	8/16 (50%)
52	14/14 (100%)	3/13 (23%)	4/11 (36%)	2/12 (17%)
76	13/14 (93%)	3/14 (21%)	3/15 (20%)	1/13 (8%)

*Source: Rueber and Glover, 1967

times their starting weights and males weighed four times their starting weights.

At sacrifice complete necropsies were done. All organs were examined histologically, including such tissues as diaphragm, tongue, and skeletal muscle. Special staining was done for glycogen, mucin, connective tissue, ceroid, canaliculi, hemosiderin, and lipid.

The males given injections at 52 weeks of age had more hyperplastic lesions than the other males. Six of 14 rats (43 percent) had hyperplastic nodules with one having a small hepatic carcinoma. The only other males with nodules, 2/11 (18 percent) were the 24-week-old rats. The remaining 52-week-old rats, and all except for one of the 24-week-old rats, had hyperplasia of the liver. Hyperplasia developed in less than half of the 12-week-old rats, whereas most of the 76-week-old rats had hyperplasia. Hyperplastic lesions and hyperplasia were not observed in control male rats.

The 24- and 52-week-old females had more hyperplastic nodules than did the younger females. The most striking lesions were in the 24-week-old rats. In this group, 8/10 rats (80 percent) had hyperplastic nodules and one rat had a small carcinoma of the liver. There were more hyperplastic nodules per liver and larger lesions in the females than in the males. The lesions in the 76-week old female rats were similar to those in the male rats. Lesions were not present in control female rats.

There were two kinds of hyperplastic lesions in the liver, one located in the periportal region and the other around central veins. Cirrhosis varied from mild to severe, but was unrelated to the hyperplastic lesions in individual rats. The severity and the histologic pattern of the cirrhosis were related to age and sex. Hyperplastic nodules are accepted by many investigators as being preneoplastic. If the study had been continued for a longer period of time, the hyperplastic nodules could thus have become overt tumors. Results of this study are given in Table 7.

In summary, 24- and 52-week-old rats of both sexes given subcutaneous carbon tetrachloride developed more hyperplastic hepatic nodules, as well as an occasional early carcinoma of the liver, than did rats of other ages. The number of hyperplastic lesions per liver and the size of lesions were larger in females than in males. Four-day-old rats died with necrosis of the liver and kidney.

Rueber and Glover (1970) gave subcutaneous injections to Japanese, Osborne-Mendel, Wistar, Black Rat, and Sprague-Dawley stocks of male rats 12-weeks-old. The injections were of a 50 percent solution of CCl_4 and corn oil, two times per week. The dosage was 1.3 ml/kg of body weight. There were 12 to 17 rats in each treatment group and 12 of each stock in the control groups. Control rats were given corn oil.

Rats were killed when they became moribund. Surviving controls for each strain were killed when the last experimental rat was killed. Complete necropsies were done. Special staining was done for glycogen, mucin, connective tissue, ceroid, hemosiderin, bilirubin, lipid, and fibrin.

Japanese rats survived an average of 47 weeks and Osborne-Mendel rats for 44 weeks. Black Rats and Sprague-Dawley rats were

TABLE 7

Lesions of the Liver in Male Rats Given Subcutaneous Carbon Tetrachloride*

Age (Weeks)	Hyperplasia	Hyperplastic Nodules	Carcinoma	Total Nodules Plus Carcinoma
4	6/14 (43%)	0/14 (0%)	0/14 (0%)	0/14 (0%)
12	4/11 (36%)	0/11 (0%)	0/11 (0%)	0/11 (0%)
24	8/11 (73%)	2/11 (18%)	0/11 (0%)	2/11 (18%)
52	7/14 (50%)	6/14 (43%)	1/14 (7%)	7/14 (50%)
76	10/12 (83%)	0/12 (0%)	0/12 (0%)	0/12 (0%)

Lesions of the Liver in Female Rats Given Subcutaneous Carbon Tetrachloride*

Age (Weeks)	Hyperplasia	Hyperplastic Nodules	Carcinoma	Total Nodules Plus Carcinoma
4	4/11 (36%)	0/11 (0%)	0/11 (0%)	0/11 (0%)
12	5/11 (45%)	3/11 (27%)	0/11 (0%)	3/11 (27%)
24	1/10 (10%)	8/10 (80%)	1/10 (10%)	9/10 (90%)
52	4/11 (36%)	6/11 (54%)	1/11 (9%)	7/11 (64%)
76	10/13 (77%)	2/13 (15%)	0/13 (0%)	2/13 (15%)

*Source: Rueber and Glover, 1967

dead in an average of 11 and 13 weeks. The average survival of Wistar strain rats was between these two extremes, at 33 weeks. Rats with severe cirrhosis usually died with recent hemorrhage into the stomach and/or small intestine, as well as ascites.

The strains of rats could be divided into three distinct groups by survival time, severity of cirrhosis, and the development of carcinomas of the liver. Carcinomas of the liver were present in 12/15 Japanese male rats (80 percent), 8/13 Osborne-Mendel male rats (62 percent), and 4/12 Wistar rats (33 percent), whereas Black Rat and Sprague-Dawley rats did not develop carcinomas, possibly due to their short survival. Some rats which did not have carcinomas had hyperplastic, hepatic nodules.

The cirrhosis was most severe in rats surviving for a short time, i.e., Black Rat and Sprague-Dawley. Japanese and Osborne-Mendel strains tended to have mild or moderate cirrhosis. The degree of cirrhosis in Wistar rats again was somewhere between that of the other two groups. One-half of the animals developed severe cirrhosis; the remaining had moderate cirrhosis. Carcinomas of the liver developed with mild or moderate, rather than severe, cirrhosis.

Small carcinomas were less than 5 mm in diameter. Large carcinomas measured between 1.2 and 3.1 cm. The first large carcinoma was observed after 68 weeks.

The carcinomas were usually well-differentiated, hepatocellular carcinomas in which the cells retained characteristics of normal parenchymal cells. Poorly differentiated, hepatocellular carcinomas had smaller cells, with basophilic cytoplasm. The cells in the undifferentiated carcinomas were unlike hepatic parenchymal cells. They varied in size and shape. The nuclei were large and the cytoplasm was basophilic. The well-differentiated carcinomas were observed in Japanese, Osborne-Mendel, and Wistar strains; however, the less-differentiated carcinomas were seen only in Japanese rats. There were small metastases in the lung from a well-differentiated carcinoma in one Japanese rat.

Hepatic vein thrombosis was noted in two Osborne-Mendel and two Japanese rats. Cholangiofibrosis was seen in four Osborne-Mendel rats and one of the Japanese strain.

Spleens were enlarged. Hemangiomas of the spleen were present in two Japanese rats and in one of the Osborne-Mendel strain. Atrophy of the testes, prostate, and seminal vesicles was proportionate to the degree of cirrhosis of the liver. There were carcinomas of the thyroid gland in three Osborne-Mendel and three Japanese rats, and one Japanese rat had a subcutaneous leiomyosarcoma. Two Osborne-Mendel and three Japanese rats had chronic renal disease.

In summary, the development of carcinomas of the liver in rats given subcutaneous injections of CCl_4 was inversely related to the severity of cirrhosis and survival time. It appeared that Sprague-Dawley, Black Rat, and, to a lesser extent, Wistar male rats died from moderate or severe cirrhosis before they could develop carcinomas of the liver. Japanese and Osborne-Mendel male rats, on the other hand, were less susceptible to the development of cirrhosis; they survived for a long period and had hepatocellular carcinomas of the liver; one had metastases to the lung (Table 8).

TABLE 8

Cirrhosis, Hyperplastic Nodules, and Hepatic Carcinomas in Male Rats Given Subcutaneous Carbon Tetrachloride*

Strain	Hyperplastic Nodules	Carcinomas	Cirrhosis			
			Mild	Moderate	Severe	Total
Japanese	3/15	12/15 (80%)	9	5	1	15/15
Osborne-Mendel	4/13	8/13 (62%)	2	7	4	13/13
Wistar	7/12	4/12 (33%)	0	6	6	12/12
Black Rat	7/17	0/17 (0%)	0	4	13	17/17
Sprague-Dawley	2/16	0/16 (0%)	0	0	16	16/16

*Source: Rueber and Glover, 1970

Cameron and Karunaratne (1936) looked at CCl_4 cirrhosis in relation to liver regeneration in the rat. Albino rats weighing about 150 g each were injected subcutaneously with 0.1 to 0.25 ml carbon tetrachloride twice a week.

After 6 to 10 doses, changes which may have developed in the liver disappeared within 7 to 10 days after cessation of treatment. With longer periods of exposure, the liver showed less and less tendency to return to a normal appearance when the chemical was discontinued. Cirrhosis of the liver developed after several doses and was severe and irreversible after 40 doses.

The liver was pale, tough, and finely granular. There was extensive fibrosis radiating from the portal areas, thereby dividing the liver into small irregular masses. Hyperplastic nodules were seen in different parts of the liver.

In this study, rats given subcutaneous injections of carbon tetrachloride readily developed cirrhosis of the liver. Also, there were presumably preneoplastic and hyperplastic nodules of the liver.

Rueber in 1970 performed a study similar to his previous one on the accentuation of hyperplastic and neoplastic hepatic lesions by methylcholathrene. Inbred Buffalo strain male and female rats 5, 8, 12, 24, 52, and 76 weeks old were used. Groups of rats of each age and sex were treated with either: (1) only carbon tetrachloride (CCl_4); (2) only 3-methylcholanthrene (MCA); or (3) CCl_4 and MCA simultaneously. There were 10 to 17 rats in each group.

Laboratory meal was ingested ad libitum. At a dosage of 1.3 ml/kg body weight, CCl_4 was injected subcutaneously twice weekly; 0.033 percent MCA was added to the meal.

Rats given CCl_4 and MCA simultaneously were killed when moribund or at the end of 12 weeks. Rats given only CCl_4 were killed between 6 and 12 weeks to correspond to the time of death for those receiving CCl_4 and MCA. Rats receiving only MCA, as well as control rats, were killed at the end of 12 weeks.

Complete necropsies were done, and all tissues were examined histologically. Special staining was carried out for glycogen, mucin, connective tissue, ceroid, canaliculi, hemosiderin, and lipid.

Rats given both CCl_4 and MCA lost weight, whereas those given only CCl_4 or MCA gained weight. Terminally, the male rats receiving both chemicals weighed 45 to 100 g less than those given CCl_4; the females weighed 20 to 55 g less. The controls and the rats ingesting MCA gained more weight than those on CCl_4.

Male rats of all ages treated with CCl_4 and MCA survived an average of 9.2 weeks (range, 5.7 to 12), and the females lived an average of 11.0 weeks (range, 6.2 to 12). Survival times of rats receiving CCl_4 were similar because of the experimental procedure. Rats fed MCA and the controls lived for 12 weeks.

Hyperplastic nodules were observed in rats 8, 24, and 52 weeks of age (those given only CCl_4). There was one small, hepatic carcinoma in an 8-week-old rat. Hyperplastic nodules were induced by MCA and CCl_4 in rats of all ages. Carcinomas were found in rats 12 weeks of age and older. The incidence of nodules and carcinomas increased with increasing age of the rats, with one exception. The incidence of nodules and carcinomas was lower in the 8-week-old male rats given both chemicals than in those treated with CCl_4 alone. In rats of all other ages receiving both chemicals, there were more nodules and more carcinomas per liver. In females, the total number of nodules and carcinomas increased with the age of the rats.

The incidence of hyperplastic nodules in female rats 12 to 52 weeks old that received only CCl_4 was greater than in male rats. Females 24 and 52 weeks of age developed the highest incidence of nodules. In comparison to the 5-, 8-, and 12-week-old rats, 76-week-old females were less prone to develop nodules. Males 8 weeks old were more susceptible to the growth of nodules and carcinomas than were females of the same age. One 24-week-old and another 52-week-old female had early hepatic carcinomas.

Nodules and early carcinomas increased notably in females 24, 52, and 76 weeks of age that were treated with CCl_4 and MCA simultaneously. Almost all 52- and 76-week-old rats had nodules or carcinomas of the liver. The increase in numbers of nodules and carcinomas per liver was even more striking in the females than in the males.

Control rats or rats ingesting MCA did not have hepatic lesions. Rats given injections of CCl_4 had only mild or moderate cirrhosis. Those fed MCA in the diet simultaneously with injections of CCl_4 developed severe cirrhosis. The incidence of hepatic vein thrombosis was markedly increased in rats given both chemicals, except for both 8-week-old males and females.

Cholangiofibrosis was increased in 5-week-old males and 24-week-old females treated with both chemicals.

There was a transitional cell carcinoma of the urinary bladder in one 8-week-old female rat receiving both chemicals. All results are given in Table 9.

In summary, methylcholathrene increases the incidence of hyperplastic, hepatic nodules and early carcinomas in rats of all ages. The difference was greater in rats 12 weeks of age and older. Females were more susceptible to the development of hyperplastic nodules and carcinomas than were males. Multiple nodules and carcinomas were observed in the livers of rats given both chemicals, whereas rats receiving only carbon tetrachloride had fewer lesions per liver. Cirrhosis of the liver was more advanced in rats given methylcholanthrene and carbon tetrachloride simultaneously.

A number of studies were performed utilizing mice to test for a relationship between CCl_4 exposure and carcinomas. Edwards, et al. (1942) did such an investigation. The mice used in this study were inbred strain L (their incidence of spontaneous hepatomas is extremely low). Mice were 2.5 to 3.5 months or 3.5 to 7.5 months of age at the start. The number of mice varied from 8 to 39 per group.

Carbon tetrachloride of a high degree of purity was administered in olive oil by stomach tube usually three, but occasionally two, times weekly. Each treatment consisted of 0.1 cc of a 40 percent solution or 0.04 ml of CCl_4. Mice also ingested Purina dog chow.

Mice were given 46 administrations of CCl_4 over a 4-month period and were killed and necropsied 3 to 3.5 months after the last

TABLE 9

Lesions[a] of the Liver in Male Rats Given Subcutaneous Carbon Tetrachloride and Methylcholanthrene*

Age	CCl_4				CCl_4 & MCA			
(Weeks)	HY	HN	C	Total	HY	HN	C	Total
5	4/12 (33%)	0/12 (0%)	0/12 (0%)	0/12 (0%)	9/17 (53%)	5/17 (29%)	0/17 (0%)	5/17 (29%)
8	2/12 (17%)	7/12 (58%)	1/12 (8%)	8/12 (66%)	6/15 (40%)	4/15 (27%)	0/15 (0%)	4/15 (27%)
12	4/10 (40%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	6/16 (38%)	4/16 (25%)	1/16 (6%)	5/16 (31%)
24	6/11 (54%)	1/11 (9%)	0/11 (0%)	1/11 (9%)	7/16 (44%)	3/16 (19%)	1/16 (6%)	4/16 (25%)
52	5/10 (50%)	3/10 (30%)	0/10 (0%)	3/10 (30%)	4/13 (31%)	5/13 (38%)	2/13 (15%)	7/13 (54%)
76	10/14 (71%)	0/14 (0%)	0/14 (0%)	0/14 (0%)	5/14 (36%)	5/14 (36%)	2/14 (14%)	7/14 (50%)

Lesions[a] of the Liver in Female Rats Given Subcutaneous Carbon Tetrachloride and Methylcholanthrene*

Age	CCl_4				CCl_4 & MCA			
(Weeks)	HY	HN	C	Total	HY	HN	C	Total
5	5/12 (42%)	0/12 (0%)	0/12 (0%)	0/12 (0%)	8/18 (44%)	6/18 (34%)	0/18 (0%)	6/18 (34%)
8	7/12 (58%)	3/12 (25%)	0/12 (0%)	3/12 (25%)	6/16 (38%)	5/16 (31%)	1/16 (6%)	6/16 (38%)
2	5/11 (45%)	2/11 (18%)	0/11 (0%)	2/11 (18%)	4/16 (25%)	8/16 (50%)	2/16 (13%)	10/16 (63%)
24	3/11 (27%)	5/11 (45%)	1/11 (9%)	6/11 (54%)	4/16 (25%)	7/16 (44%)	5/16 (31%)	12/16 (75%)
52	5/11 (45%)	5/11 (45%)	1/11 (9%)	6/11 (54%)	1/13 (8%)	8/13 (62%)	3/13 (23%)	11/13 (85%)
76	11/14 (79%)	2/14 (14%)	0/11 (0%)	2/11 (18%)	2/13 (15%)	6/13 (46%)	5/13 (38%)	11/13 (85%)

*Source: Rueber, 1970

[a] HY = hyperplasia; HN = hyperplastic nodule; C = carcinoma; Total = nodules plus carcinoma.

treatment. The mice varied from 8.5 to 14 months of age at necropsy. The liver was examined histologically.

Thirty-four of 73 mice (47 percent) given CCl_4 developed hepatomas. The incidence of tumors in the younger mice was essentially similar to that for the older mice, with the exception of the older females where the incidence was considerably lower. Tumors of the liver were observed in 7/15 younger male mice (47 percent), 21/39 older male mice (54 percent), 3/8 younger females (38 percent), and 3/11 older females (27 percent). Cirrhosis of the liver was not mentioned.

The tumors in mice given CCl_4 were usually multiple, appearing as gray or grayish-yellow, bulging nodules ranging from 2 to 15 mm in diameter. The tumors were not encapsulated and were not invasive. The adjacent hepatic tissue was compressed. Tumor cells closely resembled the hepatic parenchymal cells. The authors reported that: "The tumor cells were arranged in cords which alternated with endothelial-lined sinusoids...None of the tumors observed appeared to invade blood vessels, and there were no metastases" (Edwards, et al. 1942).

Hepatomas have been observed in 2/152 untreated strain L mice (1 percent). One of 23 untreated virgin male mice (4 percent) and 0 of 28 females (0 percent), necropsied at 15 months of age, had tumors of the liver. Tumors were not present in 22 males and 28 females 18 months of age or in 27 female breeders 12 to 23 months of age. One of 24 male breeders (4 percent) had a tumor. The results are summarized in Table 10.

TABLE 10

Tumors of the Liver in Male and Female Mice Receiving Carbon Tetrachloride by Stomach Tube*

Age (Months)	Males	Females
2.5 - 3.5	7/15 (47%)	3/8 (38%)
3.5 - 7.5	21/39 (54%)	3/11 (27%)
2.5 - 7.5[a]	28/54 (52%)[b]	6/19 (32%)[b]

*Source: Edwards, et al. 1942

[a] These values represent total number of tumors observed in mice in both age groups.

[b] Old control mice of this strain exhibit a very low incidence, as compared to CCl_4-treated mice. Hepatomas were present in 2/71 untreated males (3%) and 0/81 untreated females (0%).

In summary, strain L male and female mice were highly susceptible to the induction of hepatomas by carbon tetrachloride, and male mice were slightly more susceptible than female mice.

Eschenbrenner and Miller (1943) studied the effects of size and spacing of multiple CCl_4 doses in the induction of hepatomas. Strain A mice were used because of their normal low incidence of tumors of the liver in untreated mice (1 percent or less). Male and female mice were 2.5 to 3 months old at the beginning of the study. They ingested Purina dog chow pellets and tap water ad libitum.

Carbon tetrachloride was of a chemically pure quality and was diluted with U.S.P. olive oil. Solutions of CCl_4 in olive oil were administered by stomach tube. All mice received 30 doses of the solution or olive oil alone. Five dilutions of CCl_4 were used: 32, 16, 8, 4, and 2 percent solutions. Mice received 0.005 ml of solutions per g body weight containing 16×10^{-4}, 8×10^{-4}, 4×10^{-4}, 2×10^{-4}, or 1×10^{-4} ml, respectively, of CCl_4. Central necrosis of the liver was produced by each of these doses. Control mice received 0.005 ml of olive oil per g body weight.

The experimental and control groups were subdivided into 5 subgroups according to the interval between successive doses (1, 2, 3, 4, and 5 days) and the total period of treatment (29, 58, 87, 116, or 145 days). Equal numbers of male and female mice were used in each of the experimental and the five control groups. All mice were examined for the presence of hepatoma 150 days after the first dose. Some of them were killed at that time; others were subjected to laparotomy. It hepatomas were not present, laparotomies were performed at monthly intervals thereafter to determine if hepatomas eventually did appear. The gross diagnoses of hepatoma were confirmed by histological examinations.

In the lower dosage and shorter interval groups, hepatomas were few in number and small in size. With increases in dose and increase in interval between successive doses, there was a progressive increase in the number of small hepatomas and in the size of hepatomas for a given mouse. There was no difference in the incidence of tumors of the liver between males and females.

The authors distinguished between "spontaneous" and "gross" hepatomas on the basis of gross and histological characteristics. The tumors were suspected of being "spontaneous" by their yellow color, as contrasted with the pale pink color of induced hepatomas, and were distinguished from them histologically by their architecture and by the structure of their mitochondria.

In this study, the incidence of hepatomas increased with an increase in total time during which a given amount of carbon tetrachloride was administered. A given incidence of hepatomas was obtained with progressively less total amount of carbon tetrachloride as the duration of administration was increased.

Andervont and Dunn (1955) did a study to compare the transplantability of tumors of the liver and to compare the susceptibility of male mice with female mice to CCl_4. Carbon tetrachloride was administered because, in a previous study, eight tumors of the liver, induced by CCl_4, failed to grow when transplanted in new hosts.

Strain C3H mice of both sexes, three and six months of age, were used. Litter mates were divided equally between those receiving azo dye, o-aminoazotoluene (used as the control agent), and those receiving CCl_4. Carbon tetrachloride was dissolved in olive oil and administered by stomach tube. The first dose of 0.25 ml of a 4 percent solution of CCl_4 was toxic and killed a number of mice. One week later, 0.15 ml of the same concentration also proved to be toxic. In the following two weeks, the dose was lowered to 0.2 ml of a 2 percent solution, which was tolerated by the mice. Each mouse then was given, at weekly intervals, 17 treatments of 0.2 ml of a 3 percent solution. Mice given o-aminoazotoluene each received, at monthly intervals, four subcutaneous injections of 10 mg dissolved in 0.5 ml of olive oil. The mice were killed when they were 10 to 16 months old.

A portion of the hepatoma selected for transplantation was prepared for histologic study, and pieces of the remainder were implanted subcutaneously by means of the trocar technique. Thirty hepatomas from CCl_4-treated mice were transplanted, of which two failed to grow. Those that grew were carried through 4 to 6 transplant generations. In addition, 9/10 "spontaneous" hepatomas and 5/6 o-animoazotoluene hepatomas grew.

Eschenbrenner and Miller (1946) performed another study investigating liver necrosis and the induction of CCl_4 hepatomas in strain A mice. The mice ingested Purina dog chow pellets and water ad libitum. Five male and five female mice were used in each of seven different doses and two control groups. Treatment with CCl_4 was started when the mice were three months of age and was terminated when they were seven months old. Animals were necropsied at eight months. All mice were given one additional dose of the solution 24 hours prior to necropsy.

The CCl_4 was chemically pure and was diluted with U.S.P. olive oil. Solutions of 1.0, 0.5, 0.25, or 0.125 percent were administered by stomach tube based on body weight.

Mice in two groups at each dose level were administered the same total amount of CCl_4 over the same period of time, but with a variation in the number of doses into which the total amount was divided, and therefore in the size of each dose. This first group of mice per dose was given 30 doses of CCl_4 (0.02 ml solution/g body weight) at intervals of four days, whereas those in group two received 120 daily doses (0.005 ml solution/g body weight). The doses for mice in group one were previously determined as being "only necrotizing," and in group two as "necrotizing" and "non-necrotizing." Two control groups of mice received 0.02 ml or 0.005 ml of olive oil per g body weight per dose. All results are summarized in Tables 11 and 12.

Mice receiving the largest dose (1 percent solution) had multiple hepatomas up to 1 cm in diameter; those given smaller doses (0.5 or 0.25 percent solution) had two or more smaller tumors. The hepatomas were larger and greater in number in mice that received 120 doses than in those given 30 doses of a solution (in both groups the total amount of CCl_4 was the same). Gross tumors were not observed in mice given the 0.125 percent solution; however, there were early tumors on histological examination of the liver in two mice. Mice given olive oil did not have tumors.

TABLE 11

Hepatomas and Necrosis in Male Mice Given Carbon Tetrachloride by Gastric Intubations*

Dose (% Solution)	120 Doses		30 Doses	
	Necrosis	Tumor	Necrosis	Tumor
0	0/5	0/5	0/5	0/5
1/8	0/5	0/5	0/5	2/5[a]
1/4	0/5	5/5	3/5	4/5
1/2	0/5	5/5	4/4	3/4
1	0/4	4/4	---	---

*Source: Eschenbrenner and Miller, 1946

[a] Microscopic Tumors.

TABLE 12

Hepatomas in Female Mice Given Carbon Tetrachloride by Gastric Intubations*

Dose (% Solution)	120 Doses		30 Doses	
	Necrosis	Tumor	Necrosis	Tumor
0	0/5	0/5	0/5	0/5
1/8	0/5	0/5	0/5	0/5
1/4	0/5	5/5	2/5	3/5
1/2	0/5	5/5	2/4	2/4
1	0/5	5/5	---	---

*Source: Eschenbrenner and Miller, 1946

The presence or absence of hepatomas and of hepatic necrosis was determined. When necrosis of the liver was found in mice with tumors, necrosis was not observed in the hepatomas. The localization of necrosis after chronic administration of CCl_4 did not appear to follow a definite pattern, in contrast to the regular pattern of centrolobular necrosis seen after a single dose.

In summary, mice receiving "non-necrotizing" doses of CCl_4 developed as many, if not more, tumors of the liver than mice given "necrotizing" doses despite the fact that equal amounts of CCl_4 were administered. Mice given the 0.125 percent solution did not have gross tumors; most mice receiving either 0.25, 0.5 or 1 percent solution did have tumors.

The National Cancer Institute (NCI, 1976) performed a study in which $B6C3F_1$ male and female mice, 35 days of age and 50 per group, were used. Treatment by oral gavage 5 times per week occurred for 78 weeks. Surviving mice were sacrificed at 92 weeks from the start of the study. The doses of CCl_4 were 1250 or 2500 mg/kg of body weight for mice of both sexes. There were 20 control mice of each sex that were given corn oil only. Mice ingested Wayne Laboratory Blox® meal. A necropsy was performed on all mice. Complete histological examinations were carried out.

Most male and female mice treated with CCl_4 were dead by 78 weeks (see Table 13). Hepatocellular carcinomas were found in practically all mice receiving CCl_4, including those dying before termination of the test (see Table 14). The first carcinomas were observed in low dose female mice at 16 weeks, in high dose female

TABLE 13

Survival of Male Mice Treated with Carbon Tetrachloride*

Dose	Initial	78 Weeks	91-92 Weeks
Control			
Matched	20	13 (65%)	7 (35%)
Pooled	77	53 (69%)	38 (49%)
Low Dose	50	11 (22%)	0 (0%)
High Dose	50	2 (4%)	0 (0%)

Survival of Female Mice Treated with Carbon Tetrachloride

Dose	Initial	78 Weeks	91-92 Weeks
Control			
Matched	20	18 (90%)	17 (85%)
Pooled	80	71 (89%)	65 (81%)
Low Dose	50	10 (20%)	0 (0%)
High Dose	50	4 (8%)	1 (2%)

*Source: NCI, 1976

TABLE 14

Lesions of the Liver in Male Mice Treated with Carbon Tetrachloride*

Dose	Hyperplastic Nodules	Carcinomas	Total
Control			
Matched		2/19 (11%)	
Pooled		[a]5/77 (6%)	
Low Dose	0/49 (0%)	[a]49/49 (100%)	49/49 (100%)
High Dose		[a]47/48 (98%)	

[a]Data used for calculation of cancer risk in Criterion Formulation section of this document.

Lesions of the Liver in Female Mice Treated with Carbon Tetrachloride

Dose	Hyperplastic Nodules	Carcinomas	Total
Control			
Matched		1/20 (5%)	
Pooled		1/80 (1%)	
Low Dose	0/40 (0%)	40/40 (100%)	40/40 (100%)
High Dose		43/45 (96%)	

*Source: NCI, 1976

mice at 19 weeks, in high dose males at 26 weeks, and in low dose males at 48 weeks, compared to 72 weeks for pooled control males and 90 weeks for pooled control females.

Cystic endometrial hyperplasia occurred in both control and treated female mice. Thrombosis of the atrium of the heart was seen in 9 or 41 high dose female mice (22 percent), all of which died with carcinomas of the liver.

In summary, this study found carbon tetrachloride to be highly carcinogenic for liver in mice.

Edwards (1941) studied hepatomas in mice induced with CCl_4. Two-hundred and seven male C3H mice, aged 3 to 6 months, and 133 male and female strain A mice, aged 2 to 3½ months, were used. They were given 0.1 ml of a 40 percent olive oil solution of carbon tetrachloride (0.04 cc CCl_4) by stomach tube two or three times weekly for 8 to 16 weeks. Autopsy was performed up to 21 weeks after the last treatment.

Olive oil was administered by stomach tube in doses of 0.1 ml two or three times weekly to control male C3H and A mice from the same stock as those mice used in treated groups. Twenty-three strain C3H mice were given CCl_4 from 39 to 50 times and were killed and examined from 9 to 11 months of age. A high percentage of the treated animals developed hepatomas. Of 143 C3H mice, which varied from 6 to 10 months of age at autopsy, 126, or 88.1 percent, showed hepatomas (Table 15). Similar tumors were present in 54 of 54 strain A mice whose ages varied from 4.5 to 12 months (Table 16).

The incidence of spontaneous hepatomas in both the C3H and A strains is markedly below that of the induced tumors in the treated

TABLE 15

Incidence of Tumors in C3H Mice Ingesting Carbon Tetrachloride*

Group	Number of Mice Autopsied	Number of Mice with Hepatomas	Incidence of Hepatomas in Percent
Controls	17	0	0%
Controls w/Olive Oil	23	1	4.3%
Treated Animals (Olive oil and 0.04 ml CCl_4)	143	126	88.1%

*Source: Edwards, 1941

TABLE 16

Incidence of Tumors in Strain A Mice Ingesting Carbon Tetrachloride*

Group	Number of Mice Autopsied	Number of Mice with Hepatomas	Incidence of Hepatomas in Percent
Controls	200	1	0.5%
Controls w/Olive Oil (0.1 ml 2 or 3X weekly)	22	0	0%
Treated Animals (Olive oil and 0.04 ml CCl_4)	54	54	100.0%

*Source: Edwards, 1941

mice. Autopsies performed on 17 C3H male mice 8.5 to 9 months of age and the same stock as that used in the study failed to show any hepatic tumors.

The tumors were occasionally solitary, but generally several tumors were found in the same liver. They were soft pink, pinkish gray, grayish yellow, or yellow and varied in diameter from 2 to 20 mm. The majority protruded above the capsular surface, and a few were pedunculated. No peritoneal implants or metastases were found. There was striking microscopic similarity to the spontaneous hepatoma. The tumor cells closely resembled hepatic parenchymal cells, as in the spontaneous tumors. The pattern of the tumor was that of epithelial cords, two cells thick, alternating with endothelial-lined blood sinuses. The hepatomas were usually sharply circumscribed, through not encapsulated, and there was no invasion of intrahepatic blood vessels.

Confer and Stenger (1966) studied nodules in the livers of C3H mice after long-term CCl_4 administration. Twenty-five male mice, five weeks of age, received rectal installations of 0.1 ml of a 40 percent solution of carbon tetrachloride dissolved in olive oil two times a week for 20 to 26 weeks. Ten control mice were given only olive oil. Fourteen mice were killed nine days after the last treatment, and the remaining mice were killed at periods of 3 to 37 weeks. The livers were examined by light and electron micropsy.

Five of the 14 mice (36 percent) killed after nine days, and 8 of 11 mice (73 percent) killed later, developed hyperplastic hepatic nodules. Cirrhosis was not observed in the liver. The nodules were grossly pale pink or white, and measured 4 to 14 mm in diameter. Histologically, the cells were uniform in size and shape.

In summary, mice given carbon tetrachloride by rectal instillation had hyperplastic nodules that persisted after the discontinuation of the chemical, but did not develop cirrhosis of the liver. Such hyperplastic nodules are precursors of carcinoma of the liver.

In 1942, using CCl_4, Edwards and Dalton (1942) studied the induction of cirrhosis of the liver and hepatomas in mice. They investigated the outcome of high dose, low dose, and limited treatment.

For high dose administration, strain C3H male mice, male and female strain A mice, and strain C female mice were used. The mice ingested Purina dog chow. They were started on the study when 1 to 5 months of age.

The carbon tetrachloride contained no impurities. A dose of 0.1 ml of a 40 percent solution of CCl_4 in olive oil was administered by stomach tube two or three times per week. The number of treatments varied from 23 to 58, but a number of mice were killed after receiving 1 to 23 doses in order to study the early pathologic changes. In another study, male mice were given 0.1 ml of olive oil two or three times a week for 39 to 62 doses.

Animals were killed at one year of age or younger by cervical dislocation. Subcutaneous transplants of tumor tissue were made by the trocar technique into mice of homologous strains. Special histological techniques were used to examine a number of primary and transplanted tumors. These include techniques for the presence of fat, glycogen, or alkaline phosphatase and those for studying the mitochondria and Golgi apparatus.

Hepatomas were observed in 88 percent of C3H male mice treated with CCl_4, whereas they occur in 4 percent of untreated mice of the same age and strain. Tumors of the liver developed in 60 percent of male and female Y strain mice, whereas only 2 percent were seen in untreated mice of that strain. Liver tumors were seen in 98 percent of strain A mice of both sexes, whereas only 2 percent of these mice develop the tumor spontaneously. Hepatic tumors were found in 83 percent of C strain females, compared with 0 percent of untreated mice of the same age and strain. Results of both the treated and controls are given in Tables 17 and 18.

The hepatic tumors observed in this study were usually multiple - as many as 10 occurring in one liver. They varied from 0.1 to 2.0 cm in diameter, and there was some conciliation between the size of the tumor and the duration. The smaller tumors were reddish gray and bulged above the hepatic capsule. The larger tumors were soft, either gray or yellow, and a few of these were pedunculated and hung suspended from the liver by a pedicle of shrunken hepatic tissue; there also was cirrhosis of the liver.

Microscopically, the tumors were non-encapsulated, well-differentiated hepatomas compressing the adjacent hepatic tissue. The tumor was made up of cords, often one or two cells with endothelial -lined sinusoids. The cells resembled hepatic parenchymal cells from which they could usually be distinguished by their faintly basophilic cytoplasm. The hepatoma cell varied

TABLE 17

Hepatomas in Male and Female Mice Given Carbon Tetrachloride by Stomach Tube[*]

Strain	Age(months)	Males	Females	Both
C3H	6-10	126/143 (88%)	-	-
Y	4-12	-	-	9/15 (60%)
C	6-7	-	34/41 (83%)	-
A	4-12	-	-	161/164 (98%)

Hepatomas in Untreated Male and Female Mice

Strain	Age(months)	Males	Females	Both
C3H	8-11	2/50 (4%)	-	-
C3H	12-19	86/320 (27%)	-	-
Y	10-16	-	-	3/129 (2%)
C	13-24	-	0/150 (0%)	-
A	4-8	-	-	0/400 (0%)
A	12-16	-	-	8/400 (2%)

[*] Source: Edwards and Dalton, 1942

TABLE 18

Hepatomas in Male Mice Given Olive Oil by Stomach Tube*

Strain	Age(months)	Incidence
C3H	10-11	4%
C	12	0%
A	5-12	0%

*Source: Edwards and Dalton, 1942

considerably in size. In many it was larger and in few it was smaller than the normal hepatic cell.

Invasion of blood vessels by hepatomas was not seen, even though rather wide blood vessels were often in contact with the periphery of the tumor nodules. Metastases were not observed.

Tumors did not appear to have been induced in any of the other organs. The hepatoma that successfully grew on transplantation was well differentiated; the subcutaneous transplants that resembled the primary tumor were invasive.

Several special microscopic techniques were used to study a number of primary and transplanted tumors. Much fat and glycogen was present as large droplets in primary and transplanted tumor cells. There was little or no alkaline phosphatase.

Low dose administration (0.1 ml of 5 percent CCl_4 in olive oil - 0.005 ml) was administered three times weekly by stomach tube to 58 strain A female mice, 2.5 months of age, for 2 months. Mice were necropsied 2 days to 4.5 months after the last treatment. Hepatomas were present in 41 mice (71 percent), and some mice had cirrhosis of the liver.

The total dose (0.125 to 0.145 ml of CCl_4) is comparable to the total dose of 0.120 ml of CCl_4 in the study in which mice were given treatments of 0.04 ml each. The tumors of the liver were similar in both studies.

Limited treatment involved strain A female mice, two months of age. There were 21 to 62 mice in three treatment groups. The CCl_4 used was dissolved in olive oil, the volume of the mixture administered amounting to 0.1 ml. The mice were given 1 to 3 treatments.

The doses, which were hepatotoxic, were 0.04, 0.01, or 0.005 ml of CCl_4. Eleven mice received olive oil only. The mice were necropsied 2 to 12 months after the start of the study.

Tumors of the liver were not found in these mice. There was pigment in Kupffer cells, foci of basophilic debris, and an increase in connective tissue and reticulum.

In summary, carbon tetrachloride induced significant numbers of tumors of the liver, as well as cirrhosis, in three strains of mice. The neoplasms were similar to those induced in mice by another hepatic carcinogen, o-aminoazotolene.

Since successful transplantation is frequently considered to be a criterion of neoplasia, Leduc and Wilson (1959) attempted to transplant CCl_4-induced tumors of the liver in mice. At first "numerous failures to establish a transplantable CCl_4-induced hepatoma supported the idea that, if transplantability is a criterion, the nodules might be hyperplastic but not neoplastic. Subsequently, however, several such hepatomas were successfully transplanted from a host that was allowed to live for a long period after the CCl_4 administration ceased" (Leduc and Wilson, 1959).

Male mice of the BUB strain were used. Spontaneous hepatomas have not been found in this strain, which is now in its 40th generation. The mice ingested Purina Laboratory Chow.

Carbon tetrachloride was administered by stomach tube in doses of 0.1 of a 40 percent solution in olive oil (0.04 ml of CCl_4) per treatment. Carbon tetrachloride was given three times a week for a total of 45 to 66 doses. About one third of the mice were given three daily intravenous injections of 0.2 ml of thorotrast before CCl_4 administration was started.

The first-generation tumor transplants were made subcutaneously. Subsequently, both subcutaneous and intrasplenic transplants were made. Under light ether anesthesia, implants of tumor into the spleen were made by an incision through the dorsal body wall. The spleens were examined periodically by laparotomy.

Hepatomas did not develop in 20 control mice given thorotrast only. Hepatomas did occur in CCl_4-treated mice that were free of thoratrast.

The CCl_4 hepatomas (5 of 7) that were successfully transplanted differed from those that did not grow in new hosts in previous studies because a longer time period elapsed between CCl_4 administration and tumor transplantation. The five successful transplants were obtained from a single host killed 8 months after the last treatment, whereas those that did not grow were transplanted 11 weeks or so after the last treatment.

The authors note that:

> Chronic CCl_4 injury to the liver induces the development of both hyperplastic nodules and hepatomas, and the livers of our CCl_4-treated mice were conspicuously cirrhotic with numerous hyperplastic nodules. The nodules selected as tumors differed from the rest principally in size; histologically, there was little if any difference, and it is possible that the hepatomas which we transplanted 11 weeks or less after CCl_4 treatment was (sic) not far removed from the hyperplastic state. Thus, this growth must have been dependent on the particular conditions which were lacking in the normal mice that were recipients of the tumor implants.

They concluded:

> This suggests that there is a progressive increase in the capacity for autonomous growth in the primary hepatoma. This is based on the observation that the primary hepatomas were more readily transplanted when they had a long sojourn in the host.

Della Porta, et al. (1961) orally administered carbon tetrachloride to Syrian golden hamsters as a part of a larger investigation of the response of this species to carcinogens that induced neoplasms of the liver in other species. Ten female and 10 male Syrian golden hamsters, 12 weeks old, were used. Males weighed an average of 109 g and females 99 g. They were housed in plastic cages on wood shavings in groups of five and were given Rockland diet in pellets and tap water ad libitum. The treatment consisted of weekly administration by stomach tube of a 5 percent solution of CCl_4 in corn oil for 30 weeks. During the first seven weeks, 0.25 ml of the solution containing 12.5 µl of CCl_4 was given each week. This dose was then reduced to 0.125 ml and contained 6.25 µl of CCl_4. After this treatment, the survivors were kept under observation for 25 additional weeks and then killed.

Detailed histopathological examinations of all hamsters were conducted, except for one female lost through cannabalism at the 28th week.

Weights of the hamsters varied irregularly during the period following treatment. In general, the weights increased. Females weighed an average of 114 g and males 118 g.

One female died at the 10th week of treatment; three females and five males died or were killed between the 17th and the 28th week. Three females died at weeks 41, 43, and 54. The surviving three females and five males were killed at the end of the 55th week.

Hamsters dying during the treatment and at the 41st week had cirrhosis, as well as hyperplastic nodules that were composed of two to several layers thick; the cells showed irregularities in the shape, size, and staining qualities of their cytoplasm and nucleus, with an uneven distribution of glycogen.

All of the animals, five males and five females, dying or killed 13 to 25 weeks after the end of the treatment, had one or more liver-cell carcinomas (a total of 22 tumors: 12 in the 5 females and 10 in the males). These tumors varied in size from 4 to 30 mm and were located in all lobes: seven in the left, nine in the right, and six in the posterior lobes. Grossly, their grayish pink color, forms, and consistency differentiated them from the hyperplastic, regenerative nodules. Histologically, they were composed of atypical cells, often in mitosis, either in solid masses without any structure or arranged in small nests and in short trabeculae surrounded by dilated vascular spaces with numerous endothelial cells. Reticula on many occasions completely surrounded small nests of tumor cells. This pattern was helpful in distinguishing liver-cell carcinomas from adenomatous nodules. The tumors did not have a capsule, but compressed and invaded the surrounding parenchyma. One of the larger liver-cell carcinomas metastasized to the mesenteric and cervical lymph nodes. This tumor and six others were transplanted unsuccessfully.

The authors noted in the discussion that:

> The diagnosis of malignancy in the liver tumors observed was based on histological criteria only, to which little was added by the solitary case of metastasis. The negative result of the transplantation study deserves further investigation. Many other tumors of hamsters have been successfully transplanted to non-inbred hamsters in this and other laboratories. Leduc and Wilson (1959) suggested as an important factor the length of the interval between the last administration of carbon tetrachloride and the transplantation (Della Porta, et al. 1961).

In summary, carbon tetrachloride is a liver carcinogen in the hamster. Hyperplastic nodules (adenomatous nodules) appeared during treatment, and carcinomas appeared after CCl_4 administration had been discontinued, which strongly suggests that the nodules or benign tumors later became carcinomas. It should be noted that this study is the only report of the induction of tumors in hamsters by CCl_4.

In concluding this section, it should be noted that some of the research that has been reported suggests that hepatomas occur only after liver necrosis and fibrosis have occurred (Edwards, 1941; Edwards and Dalton, 1942; Della Porta, et al. 1961; Rueber and Glover, 1967; Reuber and Glover, 1970). The results have been interpreted to mean that "as far as the liver is concerned, hepatoma is an occasional consequence of the induction of post-necrotic cirrhosis and that CCl_4 is not a direct liver carcinogen" (Louria, 1977). The results reported by Eschenbrenner and Miller (1946), however, refute Louria's statement. These authors decided that if carbon tetrachloride is, in fact, a carcinogenic agent, tumors should be obtained with non-necrotizing doses. A series of questions concerning the mechanisms of the toxicity and carcinogenicity of carbon tetrachloride led Eschenbrenner and Miller to a series of experiments examining the issue. Their conclusions included the following:

> While it was found that a correlation exists between the degree of liver necrosis and the incidence of hepatomas in relation to dose, the use of a graded series of necrotizing and non-necrotizing doses indicated that repeated liver necrosis and its associated chronic regenerative state are probably not necessary for the induction of tumors with carbon tetrachloride (Eschenbrenner and Miller, 1946).

A list of authors addressing the issue of liver necrosis induced by carbon tetrachloride is provided in Table 19.

TABLE 19

Studies in Which Liver Cancer was Induced Using Carbon Tetrachloride

	Author	Year	Species
1.	McCord	1932	none identified -- subcutaneous injection
2.	Edwards	1941	mice -- gavage
3.	Edwards and Dalton	1942	mice -- ingestion
4.	Eschenbrenner and Miller	1946	mice -- ingestion
5.	Della Porta, et al.	1961	hamsters -- ingestion
6.	Reuber and Glover	1967	rats -- subcutaneous injection
7.	Hashimoto, et al.	1968	human -- ingestion
8.	Reuber and Glover	1970	rats -- subcutaneous injection

CRITERION FORMULATION

Existing Guidelines and Standards

At present, there is neither a water standard or an air standard for CCl_4. However, a number of standards have been recommended for inhalation in the work environment. NIOSH (1975) has summarized these standards. The following description is paraphrased from this NIOSH report.

The Sub-Committee on Threshold Limits of the National Conference of Governmental Industrial Hygienists (NCGIH) published a list in 1942 entitled, "Maximum Permissible Concentrations of Atmospheric Contaminants as Recommended by Various State Industrial Hygiene Units" (NCGIH, 1942). Thirteen states were listed as recommending 650 mg/m^3 for carbon tetrachloride in air. The listing was presented without comment, other than that the tabulated values were not to be construed as recommended safe concentrations.

Various standards for the inhalation of carbon tetrachloride were the subject of discussion at the 7th Annual Meeting of NCGIH (Bowditch, 1944). Manfred Bowditch, Director of the Massachusetts Division of Occupational Hygiene, gave "temporary indisposition," indicated by nausea, as reason for a standard lower than 650 mg/m^3. He reported that, as a consequence, the Division of Occupational Hygiene of the Massachusetts Department of Labor and Industries proposed lowering the standard for carbon tetrachloride to 260 mg/m^3. Other governmental agencies also considered 650 mg/m^3 ineffective and recommended a lower standard (Bowditch, 1944; Cook, 1945).

A list of maximum allowable concentrations of atmospheric industrial contaminants compiled by Cook (1945) included the carbon tetrachloride values of seven governmental agencies. These are presented in Table 20.

These concentrations were all recommended as allowable for prolonged exposures, usually assuming a 40-hour workweek (Cook, 1945).

In addition to tabulating these values, Cook (1945) reported 650 mg/m^3 to be an accepted or tentative value based on the work published by Smyth, et al. (1936). However, in his discussion of carbon tetrachloride, Cook (1945) wrote that since Smyth's publication there was an increasing amount of evidence of injury to health at lower concentrations, and he recommended that exposures be at less than half the 650 mg/m^3 then being used.

The American Conference of Governmental Industrial Hygienists (ACGIH) (formerly NCGIH) adopted a list of "Maximum Allowable Concentrations of Air Contaminants for 1946," prepared by the Sub-Committee on Threshold Limits (ACGIH, 1946) which, in accordance with Cook's recommendation (Cook, 1945), selected a value of 325 mg/m^3 for carbon tetrachloride.

The ACGIH Committee on Threshold Limits reported in 1949 that it had received comments from outside the Conference that a value of 325 mg/m^3 for carbon tetrachloride was too low (ACGIH, 1949). On the other hand, carbon tetrachloride was included in a list of substances for which a reduction of the limit had been suggested by members of the Conference.

TABLE 20

1975 Carbon Tetrachloride Inhalation Standards of Governmental Agencies*

Governmental Agency	MAC, mg/m^3
California Industrial Accident Commission	100
Connecticut Bureau of Industrial Hygiene	100
Massachusetts Department of Labor and Industries	50
New York State Department of Labor	75
Oregon State Board of Health	50
Utah Department of Health	100
United States Public Health Service	100

*Source: NIOSH, 1975

MAC = Maximum allowable concentration.

The ACGIH recommended a threshold limit value (TLV) of 162.5 mg/m^3 for carbon tetrachloride (ACGIH, 1953). A preface to future tables of threshold limits was adopted and defined the values as "maximum average atmospheric concentration of contaminants to which workers may be exposed for an eight-hour working day without injury to health" (ACGIH, 1953). The preface was modified in 1958 and included the statement that "they (threshold limit values) represent conditions under which it is believed that nearly all workers may be repeatedly exposed, day after day, without adverse effect" (ACGIH, 1958).

The American Standard Maximum Acceptable Concentration of Carbon Tetrachloride (ASA Z37.17-1957), published in 1957, was 162.5 mg/m^3 for exposures not exceeding 8 hours daily, with the understanding that variations should fluctuate around 65 mg/m^3 (ASA, 1957). The 162.5 mg/m^3 was understood to be a ceiling below which all concentrations were to fall. It was based partly on the animal experiments reported by Adams, et al. (1952) and partly on industrial experiences of members of that Committee.

The Documentation of Threshold Limit Values (ACGIH, 1962) referred to the reports of Adams, et al. (1952), Heimann and Ford (1941), Kazantzis and Bomford (1960), and Elkins (1942), in its support of the TLV for carbon tetrachloride of 162.5 mg/m^3. From these data, it was considered that 162.5 mg/m^3 was low enough to prevent irreversible injury (ACGIH, 1962).

At the annual meeting of the ACGIH in 1962, the Threshold Limit Committee recommended reducing the TLV for carbon tetrachloride to 65 mg/m^3 because there were "increasing indications that exposure to carbon tetrachloride at 162.5 mg/m^3 was excessive"(ACGIH, 1962).

"Permissible Levels of Toxic Substances in the Working Environment" for many countries was published by the International Labour Office (1970). The reported carbon tetrachloride standards are presented in Table 21. The USSR values (MAC) are absolute values never to be exceeded. They are set at a value which will not be expected to produce, in any exposed person, any disease or other detectable deviation from the normal. Some other countries tend to follow this value in setting their standards, while still others tend to follow the recommendations of the ACGIH. The intent is indicated from some of the standards presented in Table 21.

The most recent documentation of the threshold limit values was published by the ACGIH (1971). The reports of Heimann and Ford (1941), Elkins (1942), Barnes and Jones (1967), Kazantzis and Bomford (1960), Markham (1967), Adams, et al. (1952), and Stewart, et al. (1961), were referred to in support of the TLV of 65 mg/m^3, which had been adopted in 1962. Information that some workmen experienced nausea when average daily carbon tetrachloride exposures approached 162.5 mg/m^3, whereas no difficulties were experienced at 65 mg/m^3 (based on a personal communication to the Committee), was used as additional support for the TLV. The TLV of 65 mg/m^3 was recommended with the caution that peak exposures, even of short duration, should not exceed 162.5 mg/m^3.

The Occupational Safety and Health Administration, U.S. Department of Labor, adopted the American National Standards Institute (ANSI) standard Z37.17-1967 (ANSI, 1967) as the Federal

TABLE 21

Carbon Tetrachloride Inhalation Standards of 10 Countries*

Country	Standard mg/m^3	Qualifications
Czechoslovakia	50	Normal MAC
	250	Single short exposure
Finland	160	8 hours continuous exposure
Hungary	20	8-hour average
	100	30 minutes
Japan	10	
Poland	20	
Rumania	50	
UAR and SAR	625	
USSR	20	MAC
Yugoslavia	65	

*Source: Adapted from NIOSH, 1975

MAC = Maximum Allowable Concentration.

standard for carbon tetrachloride (29 CFR 1910.1000). This standard is 65 mg/m^3 for an 8-hour TWA exposure, with an acceptable ceiling exposure concentration of 162.5 mg/m^3, and an acceptable maximum peak above the acceptable ceiling concentration for an 8-hour shift of 1,300 mg/m^3 for 5 minutes in any four hours.

This ANSI standard was based on human experience and extensive studies on animals. References cited to support it were Adams, et al. (1952), Stewart, et al. (1961, 1965), Stewart and Dodd (1964), von Oettingen (1964), and Irish (1963).

Finally, a standard decided upon by the FAO/WHO Expert Committee is 50 µg/kg for cooked cereal products.

Current Levels of Exposure

A brief review of some of the data presented in the exposure section of this report will summarize the current levels of human exposure. Carbon tetrachloride has been found in some waters. An EPA survey of drinking water in the U.S. revealed that 10 percent of the supplies surveyed had 2.4 to 6.4 µg/l CCl_4.

Carbon tetrachloride (CCl_4) has been found in a variety of food-stuffs ranging from 1 to 20 µg/kg. Residues have been found in commercially fumigated wheat, corn, and milo in amounts ranging from 2.9 to 20.4 mg/kg after storage for 1 to 3 hours. Carbon tetrachloride residues ranging from 20 to 62 mg/kg in sacks of wheat were found by Wit, et al. (1972), following fumigation with a mixture of CCl_4-EDC-EDB (10.2:8:1 by weight) and then aerated for several weeks. Residues as high as 72.6 mg/kg after 1 week of aeration were detected in wheat by Berck (1974). After 7 weeks of aeration, 3.2 mg/kg were found. Flour made from this wheat had residues of 0.20 to 0.93 mg/kg. Amounts of CCl_4 detected in bread made from this wheat ranged from 0.04 to 0.13 mg/kg for wheat aerated for 3 days and 0.01 to 0.2 mg/kg for wheat aerated for 7 weeks.

The most extensive measurements of CCl_4 have occurred in the atmosphere. Virtually no variation has been found between land and ocean, urban and rural, or Northern and Southern Hemispheres. The maximum value detected was 0.117 mg/m^3 in Bayonne, N. J.; however, normal background levels range from 0.00078 to 0.00091 mg/m^3 in the continental and marine air masses.

The National Research Council (1978) in its assessment of "Nonfluorinated Halomethanes in the Environment" estimated total human exposure to CCl_4. Using drinking water concentrations of less than 2.0 to 3.0 µg/l and other conventional assumptions with regard to human and environmental conditions, three ranges of exposure were estimated (See Table 22).

Minimum, typical, and maximum exposure estimates of total CCl_4 uptake were 4.54 mg/year, 7.70 mg/year, and 629 mg/year, respectively. The percentage from fluid sources (water) was 16 percent, 23 percent, and 0.6 percent, respectively. By far the highest uptake of CCl_4 was estimated to come from atmospheric sources, 62 to 98 percent.

Although monitoring has provided more information on CCl_4 presence in the environment than most chemicals, there remain many relative unknowns about absorption, synergism/antagonism, etc. The estimated CCl_4 exposure from food sources is based upon only limited information which is compared to air and fluid uptakes. At face

TABLE 22

Relative Human Uptake of Carbon Tetrachloride (CCl_4) from Environmental Sources (mg/year)[*]

At Minimum Exposure Levels[a]

	Adult Man	
Source	CCl_4	$CHCl_3$
Fluid Intake	0.73	0.037
Atmosphere	3.60	0.41
Food Supply	0.21	0.21
Total	4.54	0.66

At Typical Exposure Levels[b]

	Adult Man	
Source	CCl_4	$CHCl_3$
Fluid Intake	1.78	14.90
Atmosphere	4.80	5.20
Food Supply	1.12	2.17
Total	7.70	22.27

At Maximum Exposure Levels[c]

	Adult Man	
Source	CCl_4	$CHCl_3$
Fluid Intake	4.05	494
Atmosphere	618	474
Food Supply	7.33	16.4
Total	629.38	984.4

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[a] Minimum conditions of all variables assumed: Minimum exposure-minimum intake for fluids; minimum exposure-minimum absorption for atmosphere; and minimum exposure-minimum intake for food supplies.

[b] Typical conditions of all variables assumed.
For CCl_4: 0.0025 mg/l-reference man intake for fluids; average of typical minimum and maximum absorption for atmosphere; and average exposure and intake for food supplies.
For $CHCl_3$: median exposure-reference man intake for fluids; average of typical minimum and maximum absorption for atmosphere; and average exposure and intake for food supplies.

[c] Maximum conditions of all variables assumed: maximum exposure-maximum intake for fluids; maximum exposure-maximum absorption for atmosphere; and maximum exposure-maximum intake for food supplies.

[*] Source: National Research Council, 1978

value, the figures indicate that while none of the three routes of exposure is negligible, inhalation is the most important for CCl_4.

Special Groups at Risk

As a result of the studies performed on animals it appears as though older animals are more susceptible to the toxic effects of CCl_4 than are younger animals (Rueber and Glover, 1967). Also, male animals are more susceptible than females (Rueber and Glover, 1967). Chaturvedi (1969) examined age and sex as factors of CCl_4 toxicity. The findings revealed that female rats are less susceptible to the ill-effects of different hepatotoxic agents and fare better than males because of different hormonal and enzyme patterns and the lack of certain proteins in contrast to the male liver. The sex difference noticed in adult rats was not so apparent in young rats.

The synergistic effects of alcohol must also be noted. Alcoholics have a greater susceptibility to poisoning from CCl_4. As described by Moon (1950), the frequent occurrence of a history of alcoholism in cases of fatal CCl_4 poisoning indicates a synergistic nephrotoxic, as well as hepatotoxic, effect between alcohol and CCl_4.

Finally, as previously mentioned, very obese and undernourished persons suffering from pulmonary diseases, gastric ulcers or a tendency to vomiting, liver or kidney diseases, diabetes or glandular disturbances are especially sensitive to the toxic effects of CCl_4.

Basis and Derivation of Criteria

Studies indicate that CCl_4 has a full spectrum of toxic effects. Industrial and accidental exposures to CCl_4 by ingestion, inhalation, and dermal routes historically have produced acute, subacute, and chronic poisoning, some of which were fatal. Acute toxicity for man and animals can be characterized generally as nodular hyperplasia and cirrhosis of the liver and renal dysfunction. Mutagenic effects have not been observed and teratogenic effects have not been conclusively demonstrated.

The most significant effect to consider in terms of dose/response is the cancer-causing potential of the chemical. Current knowledge leads to the conclusion that carcinogenesis is a nonthreshold, nonreversible process. The nonthreshold concept implies that many tumors will be produced at high doses, but any dose, no matter how small, will have the probability of causing cancer. Even small carcinogenic risks have a serious impact on society when the exposed population is large, because it is likely that some cancers will be caused by exposure to CCl_4. The nonreversible concept implies that once the tumor growth process has started, growth will continue and may metastasize and involve other organs until death ensues.

There is sufficient evidence to conclude that CCl_4 is a carcinogen in laboratory animals and, with appropriate assumptions, is interpreted to be a suspect human carcinogen.

Under the Consent Decree in NRDC vs. Train, criteria are to state "recommended maximum permissible concentrations (including where appropriate, zero) consistent with the protection of aquatic organisms, human health, and recreational activities." Carbon tetrachloride is suspected of being a human carcinogen. Because there is no recognized safe concentration for a human carcinogen, the appropriate concentration of carbon tetrachloride in water for maximum protection of human health is zero.

Because attaining a zero concentration level may be infeasible in some cases and in order to assist the Agency and states in the possible future development of water quality regulations, the concentrations of carbon tetrachloride corresponding to several incremental lifetime cancer risk levels have been estimated. A cancer risk level provides an estimate of the additional incidence of cancer that may be expected in an exposed population. A risk of 10^{-5} for example, indicates a probability of one additional case of cancer for every 100,000 people exposed; a risk of 10^{-6} indicates one additional case of cancer for every million people exposed, and so forth.

In the Federal Register (44 FR 15930) notice of availability of draft ambient water quality criteria, EPA stated that it is considering setting criteria at an interim target risk level of 10^{-5}, 10^{-6}, or 10^{-7}, as shown in the following table.

Exposure Assumptions (per day)	Risk Levels and Corresponding Criteria (1)		
	10^{-7}	10^{-6}	10^{-5}
2 liters of drinking water and consumption of 6.5 g fish and shellfish. (2)	0.04 µg/l	0.40 µg/l	4.0 µg/l
Consumption of fish and shellfish only.	0.69 µg/l	6.94 µg/l	69.4 µg/l

(1) Calculated by applying a linearized multistage model, as discussed in the Human Health Methodology Appendices to the October, 1980 Federal Register notice which announced the availability of this document, to the animal bioassay data presented in Appendix I and in Table 14. Since the extrapolation model is linear at low doses, the additional lifetime risk is directly proportional to the water concentration. Therefore, water concentrations corresponding to other risk levels can be derived by multiplying or dividing one of the risk levels and corresponding water concentrations shown in the table by factors such as 10, 100, 1,000, and so forth.

(2) Approximately 6 percent of the carbon tetrachloride exposure results from the consumption of aquatic organisms which exhibit an average bioconcentration potential of 18.75-fold. The remaining 94 percent of carbon tetrachloride exposure results from drinking water.

Concentration levels were derived by assuming a lifetime exposure to various amounts of carbon tetrachloride: (1) occurring from consumption of both drinking water and aquatic life grown in waters containing the corresponding carbon tetrachloride concentrations; and (2) occurring solely from consumption of aquatic life grown in the waters containing the corresponding carbon tetrachloride concentrations. Although total exposure information for carbon tetrachloride is discussed and an estimate of the contributions from other sources of exposure can be made, these data will not be factored into ambient water quality criteria formulation until additional analyses can be made. The criteria presented, therefore, assume an incremental risk from ambient water exposure only.

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APPENDIX I

Derivation of Criterion for Carbon Tetrachloride

Carbon tetrachloride has been studied extensively and administered orally in a number of studies in mice, rats, hamsters, and dogs. However, in these studies, either the length of the study was too short or the dose level was too high for a dose-response estimation of lifetime exposure (NRC, 1978). The National Research Council recognizes this problem and for this reason uses the NCI (1976) bioassay for trichloroethylene in determining a carcinogenic risk estimate for carbon tetrachloride.

This NCI study uses carbon tetrachloride as the positive control. Male mice receiving carbon tetrachloride by gavage at 1250 mg/kg 5 days per week for 78 weeks developed incidences of liver tumors at 92 weeks, when the experiment was terminated, as shown below. The parameters of the extrapolation model are:

Dose (mg/kg/day)	Incidence (no. responding/no. tested
0	5/77
1250 x 5/7 = 893	49/49
2500 x 5/7 = 1786	47/48

le = 78 weeks

Le = 92 weeks

L = 92 weeks

w = 0.028 kg

R = 18.75 l/kg

With these parameters the carcinogenic potency factor for humans, q_1^*, is 0.08275 $(\text{mg/kg/day})^{-1}$. The result is that the water concentrations should be less than 4.0 µg/l in order to keep the individual lifetime risk below 10^{-5}.